Anti-Infectives Advisory Committee Meeting

Briefing Document

Raxibacumab

Treatment of Inhalation Anthrax

BLA 125349

21 September 2009

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List of Abbreviations

AE	adverse event
AGM	African green monkey
ANC	absolute neutrophil count
ANOVA	analysis of variance
ALT	alanine aminotransferase
ATR	anthrax toxin receptor
AUC	area under the drug concentration-time curve
$AUC_{0\text{-}4d}$	area under the serum concentration time curve for the 4 days following dose administration
$AUC_{0\text{-}\infty}$	area under the drug concentration-time curve from time 0 to infinite time
AVA	anthrax vaccine adsorbed
BARDA	Biomedical Advanced Research and Development Authority
BBRC	Battelle Biomedical Research Center
BDS	bulk drug substance
BLA	biologics license application
BL3	Biosafety Level 3
ºC	degrees Celsius
cAMP	cyclic adenosine monophosphate
CDC	Centers for Disease Control and Prevention
CFU	colony-forming unit
CHO-K1	Chinese hamster ovary K1 cells
CHX-A”	2-(*p*-isothiocyanato-benzyl)-cyclohexyl-diethylenetriaminepentaacetic acid
CI	confidence interval
CK	creatine phosphokinase
CL	clearance
C_{max}	maximum serum (or plasma) drug concentration
CMC	chemistry, manufacturing, and controls
CMG2	capillary morphogenesis gene 2
CPK	creatine phosphokinase
CRF	case report form
CV%	percent coefficient of variation
EF	edema factor
ELISA	enzyme-linked immunosorbent assay
EUA	Emergency Use Authorization
°F	degrees Fahrenheit
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
GD	gestational day
GLP	Good Laboratory Practice
HGS	Human Genome Sciences, Inc.
HHS	Department of Health and Human Services
IC_{50}	median inhibitory concentration
ICH	International Conference on Harmonisation
IM	intramuscular(ly)

IND	Investigational New Drug application
ISS	integrated safety summary
ITT	intention-to-treat
IV	intravenous(ly)
ka	on-rate
kDa	kilodalton
Kd	equilibrium binding constant
kd	off-rate
kg	kilogram
LD_{50}	lethal dose in 50% of the test population
LF	lethal factor
mAb	monoclonal antibody
µg	microgram
mg	milligram
MIC	miniumum inhibitory concentration
mL	milliliter
mM	millimolar
NA	not applicable
ng	nanogram
NHP	non-human primate
NIH	National Institutes of Health
nM	nanomolar
NOAEL	no observable adverse-effect level
NS0	mouse myeloma cell line
NZW	New Zealand White (rabbits)
PA	protective antigen
PA63	63 kDa fragment of protective antigen
PCR	polymerase chain reaction
PD	pharmacodynamics
pg	picogram
PK	pharmacokinetic(s)
pM	picomolar
PO	orally
PT	prothrombin time
RBC	red blood cells
SAE	serious adverse event
SC	subcutaneous(ly)
SNS	strategic national stockpile
SOC	system organ class
$t_{1/2}$	terminal elimination half-life
$t_{1/2,\alpha}$	initial (alpha) phase half-life
$t_{1/2,\beta}$	terminal (beta) phase half-life
TEM8	tumor endothelial marker 8
TK	toxicokinetics
t_{max}	time of maximum serum concentration
TNA	toxin neutralizing activity

TNF	tumor necrosis factor
TTM	time to morbundity
US	United States of America
V_1	initial volume of distribution
V_{ss}	steady-state volume of distribution
WBC	white blood cells
WFI	water for injection

1 Executive Summary

Raxibacumab, the first monoclonal antibody anti-toxin for the treatment of anthrax, is the result of a coordinated response to a recognized public bioterrorism threat and the US government's request for counterterrorism measures to treat inhalation anthrax.

Following the anthrax attacks in 2001, over 30,000 people with suspected exposures initiated antimicrobial prophylaxis. Eleven people developed inhalation anthrax, and despite best available treatment, 5 of them died. All subjects received at least 2 antibiotics and some received as many as 7 (Jernigan et al, 2001). Antibiotics alone were insufficient to protect these subjects. While antibiotics can overcome bacteremia caused by antibiotic-susceptible strains of anthrax, they do not directly address the toxemia that drives pathogenesis. Anthrax toxin is responsible for the majority of the morbidity and mortality associated with anthrax. Pleural effusions, associated with the edema caused by anthrax toxin, were present in all of the subjects and required thoracentesis in the majority of survivors, often with repeat drainage and with placement of chest tubes in some.

In humans, as well as animals, inhalation anthrax occurs following inhalation of *Bacillus anthracis* (*B. anthracis*) spores, which germinate within the macrophages as they travel to the draining mediastinal lymph nodes. Multiplication of the bacteria results in a high organism count in the blood, production of bacterial toxins, and the rapid onset of septicemia. Although the bacterial replication (bacteremia) can be controlled by administration of appropriate antibiotics, it is the bacterial toxin that exerts deleterious effects on the cells within the body, resulting in substantial pathology and high mortality in infected individuals.

There is an effective anthrax vaccine that works by inducing the body's natural immune response to anthrax toxin. The vaccine confers immunity to anthrax infection by stimulating the body to produce anti-toxin antibody. Once subjects have this antibody, they are protected against the effects of anthrax. All of the survivors of the 2001 attacks eventually mounted an immune response to the anthrax toxin and developed anti-toxin antibodies by Day 28 after exposure (Quinn et al, 2004).

The challenge in this rapidly progessing and often fatal disease is the time required to generate an immune response. Anthrax vaccine requires up to 28 days to achieve protective titers of anti-toxin antibody. Once protected, a subject can be exposed to anthrax bacteria, but because the body is immune to the anthrax toxin, the subject suffers no significant morbidity or mortality from exposure to anthrax. The presence of anti-toxin antibody is protective.

Raxibacumab works by delivering human recombinant anti-toxin antibody to the subject immediately. This antibody persists long enough for the development of natural immunity to occur, helping subjects survive to go on to develop innate antibody. This immediate onset of action fills the need for subjects who have not received anthrax vaccine. This approach also addresses the need arising from the inability of antibiotics to directly address anthrax toxemia.

While it is possible to achieve 100% cure rates using antibiotics alone under experimental conditions, the 2001 attacks and other real-world experiences have demonstrated that antibiotics alone are not 100% effective. In addition, antibiotics would not be effective against

antibioitic-resistant strains of anthrax. The US government has recognized this need. They have simulated mass exposures in terror attacks, and the death tolls were of much greater magnitudes than sustained in the 2001 attacks.

In response to this danger, the US Government has made the development of a direct anti-toxin a priority for civilian defense. The Department of Health and Human Services (HHS) canvassed biotechnology and pharmaceutical companies for products for immune-based anti-toxin treatments effective against anthrax toxin. Following a joint request from the Secretaries of the Department of Homeland Security and HHS, the President approved the acquisition of anthrax therapeutics in August 2004. Project Bioshield provided a mandate and resources to foster the development of new counterterrorism measures. The Biomedical Advanced Research and Development Authority (BARDA), which manages Project Bioshield, solicited proposals for anti-anthrax therapeutics for inclusion in the Strategic National Stockpile (SNS) for the Centers for Disease Control and Prevention (CDC) to mobilize in the event of an anthrax attack.

B. anthracis produces 3 toxins, including a binding moiety, protective antigen (PA), and 2 enzymatic moieties, lethal factor (LF) and edema factor (EF) (Inglesby et al, 2002). The PA protein first binds to its cell surface receptor (TEM8 or CMG2) and is cleaved (Figure 1-1). This processing step enables the remaining fragment (PA63) to multimerize into a heptameric barrel structure and exposes a site to which LF and EF bind with high affinity. Internalization of the complex by receptor-mediated endocytosis is followed by the formation of a membrane-spanning pore. The bound EF and LF proteins are then translocated through the pore from the endosome to the cytosol where they exert their toxic effects. EF is a calmodulin-dependent adenylate cyclase which alters cellular homeostasis mechanisms, thereby resulting in edema. LF is a zinc metalloproteinase that induces a hyperinflammatory condition in macrophages resulting in the production of proinflammatory cytokines, contributing to hemodynamic alterations that progress to shock and death of infected subjects.

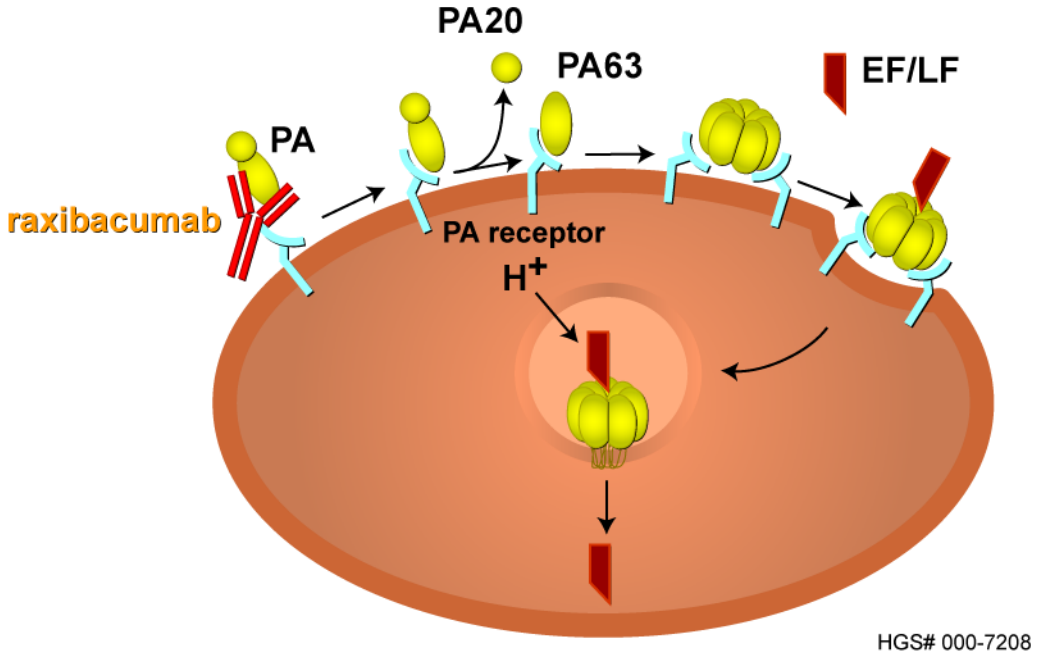

Figure 1-1 Mechanism of action of anthrax toxins and inhibition by anti-PA antibody

The protective antigen (PA) protein binds to its cell surface receptors and is cleaved by a membrane-bound furin-like protease leaving a 63 kDa fragment bound to the cell. This fragment multimerizes into a heptameric barrel structure and exposes a site on PA to which lethal factor (LF) and edema factor (EF) bind with high affinity. Internalization of this complex is followed by formation of a membrane-spanning pore. The bound EF and LF proteins are then translocated from the endosome to the cytosol where they exert their toxic effects. An antibody that binds soluble PA inhibits the anthrax toxin effects by preventing PA from binding to its receptor.

Inhibition of PA binding to its cellular receptors prevents the downstream toxin-mediated deleterious effects of the anthrax toxins. An antibody that binds and inhibits PA interaction with the cell surface provides a direct anti-toxin effect. This mechanism of action is completely different from the mechanism of action of antimicrobials, which attack the organism, causing stasis or death of the bacteria. While antimicrobials cut off the source of anthrax toxin production, they do nothing to inhibit the adverse effects of toxins that have already been released and toxemia can persist after bacteremia has been resolved. Anti-toxin antibodies directly neutralize the toxin and prevent its pathogenic effects. Recombinant human anti-toxin monoclonal antibodies immediately provide the protection that develops from the innate immune response in anthrax survivors or that is stimulated by vaccines over the course of weeks and multiple injections. Anti-toxin antibodies can be used in combination with antibiotics to protect subjects from the toxemia that antibiotics do not treat. Anti-toxin antibodies would also be an important therapeutic option when antimicrobials are unavailable, contraindicated, or in the event of exposure to an antibiotic-resistant anthrax strain.

Anthrax anti-toxin therapy is a missing element in the therapeutic armamentarium. To address the unmet need for an anthrax anti-toxin therapy, any new antibody to anthrax toxin should have several attributes:

- Bind anthrax toxin
  - with high sensitivity and specificity
  - from a broad range of *B. anthracis* strains
- Act rapidly to neutralize anthrax toxin and provide durable exposure for anti-toxin protection until innate immunity can develop
- Does not prevent innate immune response
- Does not interfere with the action of antimicrobials

Immediately after the anthrax attacks in September and October of 2001, Human Genome Sciences, Inc. (HGS) embarked on a development program to produce a monoclonal antibody to treat inhalation anthrax. In less than a year, using recombinant DNA technology, a potent and specific antibody had been developed that binds the PA of *B. anthracis* with high affinity and inhibits PA binding to anthrax toxin receptors, thus protecting animal and human macrophages from anthrax toxin-mediated cell death. HGS then began the nonclinical work to establish proof of concept of the antibody therapeutic in the laboratory and in animals and initiated the process development work to manufacture and characterize the product.

HGS designed the development program for raxibacumab to address the unmet bioterrorism and medical needs posed by inhalation anthrax and the limitations of current therapies. Raxibacumab was demonstrated to be safe and effective in the unique setting for development of a bioterrorism drug. Not only is raxibacumab the first monoclonal antibody anti-toxin against anthrax, it is the first new drug developed since the bioterrorism attacks of 2001 to seek licensure under the FDA's Animal Rule. The Animal Rule provided the regulatory structure for effectiveness to be demonstrated in animals when it is unethical or not feasible to conduct controlled clinical trials in humans. The animal studies with raxibacumab were designed to meet the 4 criteria for demonstration of efficacy under the Animal Rule:

1. use a product with a well-understood mechanism of action against a target with known pathophysiology
2. demonstrate benefit in at least 1 animal species
3. use an efficacy endpoint relevant to the desired outcome in humans
4. generate pharmacokinetic data that allow translation of effective animals exposures to recommended human doses.

The animal models also contained the essential elements provided in FDA guidance which are recommended to generate data likely to predict the effectiveness of the product in humans. The models are well characterized and the 2 animal species, New Zealand White (NZW) rabbits and cynomolgus monkeys, have been used to evaluate vaccines and antimicrobials for the treatment of inhalation anthrax. Animal models provided the effectiveness data to support the approval of ciprofloxacin and levofloxacin for the treatment of inhalation anthrax. While efficacy was demonstrated in 2 animal models of inhalation anthrax, safety was evaluated in human clinical studies with single and repeat dosing, alone and in combination with

antibiotics, in healthy adult volunteers. The animal efficacy studies demonstrated that a single 40 mg/kg dose of raxibacumab effectively neutralizes PA and significantly improves survival. Its effect is immediate, and because of its long half-life (over 20 days) is durable, maintaining anti-toxin protection until natural immunity can develop. Importantly, raxibacumab does not prevent the development of innate anti-toxin immunity in anthrax-infected animals, nor does it interfere with the activity, pharmacokinetics (PK), or safety of concomitantly administered antimicrobials.

Raxibacumab efficacy as an anthrax anti-toxin was demonstrated in well-characterized models of inhalation anthrax in NZW rabbits and cynomolgus monkeys. Single dose of 40 mg/kg raxibacumab administered to animals with symptomatic systemic inhalation anthrax, resulted in survival of 44.4% in rabbits (p = 0.0029) and 64.3% in monkeys (p = 0.0007) compared with 0% survival in placebo-treated animals (Figure 1-2). The 20 mg/kg dose of raxibacumab also produced a statistically significant, but numerically lower, increase in survival.

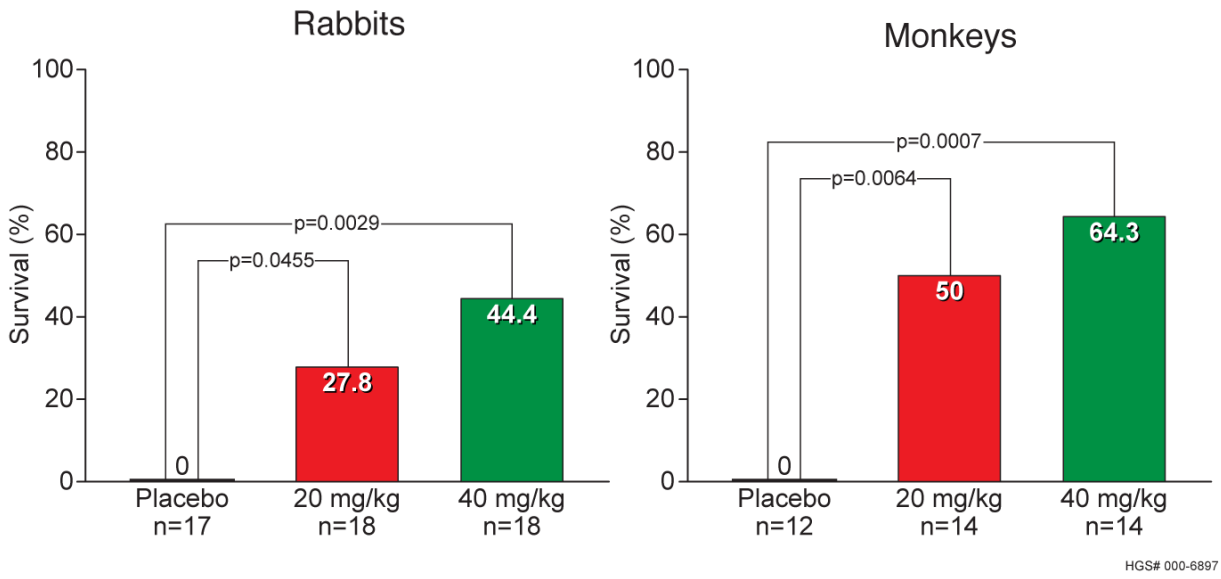

Figure 1-2 **Primary endpoint in the pivotal efficacy studies in rabbits (682-G005758) on left and monkeys (724-G005829) on right**

In both species, 20 mg/kg and 40 mg/kg raxibacumab also significantly increased survival time compared with placebo and the trend to increased survival time was dose-dependent (Figure 1-3). Increasing survival time in humans is clinically important because it would allow more time for medical intervention and for natural protective anti-toxin antibodies to develop.

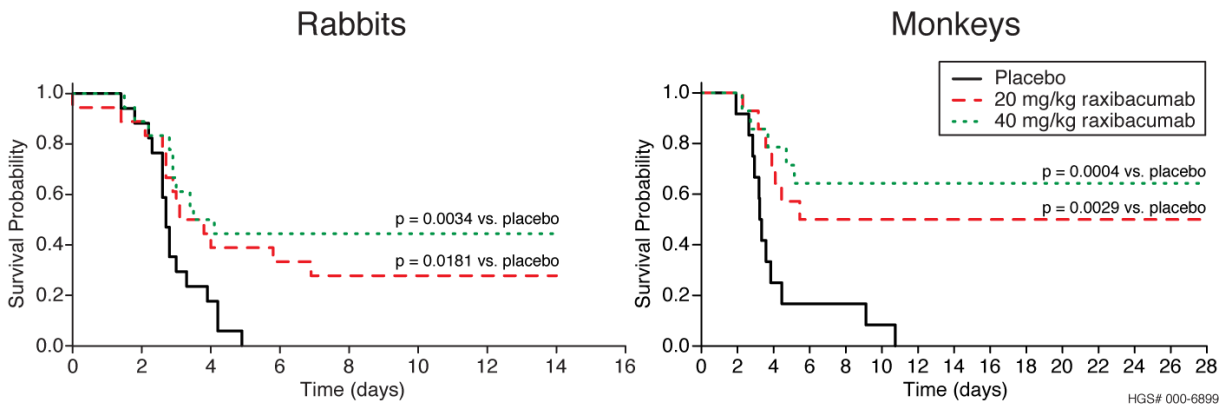

Figure 1-3 Survival time in therapeutic efficacy studies in rabbits (682-G005758) on left and monkeys (724-G005829) on right

In animals succumbing to inhalation anthrax, the serum PA profiles for rabbits and monkeys exhibited a triphasic pattern of initial rise, followed by a plateau, and then a second rise until death. In contrast, animals that were treated with 20 and 40 mg/kg raxibacumab and survived showed the initial rise, but before the plateau phase was attained, serum PA concentrations began to decline. For the surviving animals, the onset of declining serum PA concentrations was associated with attainment of negative bacteremia results and the start of normalization of clinical signs.

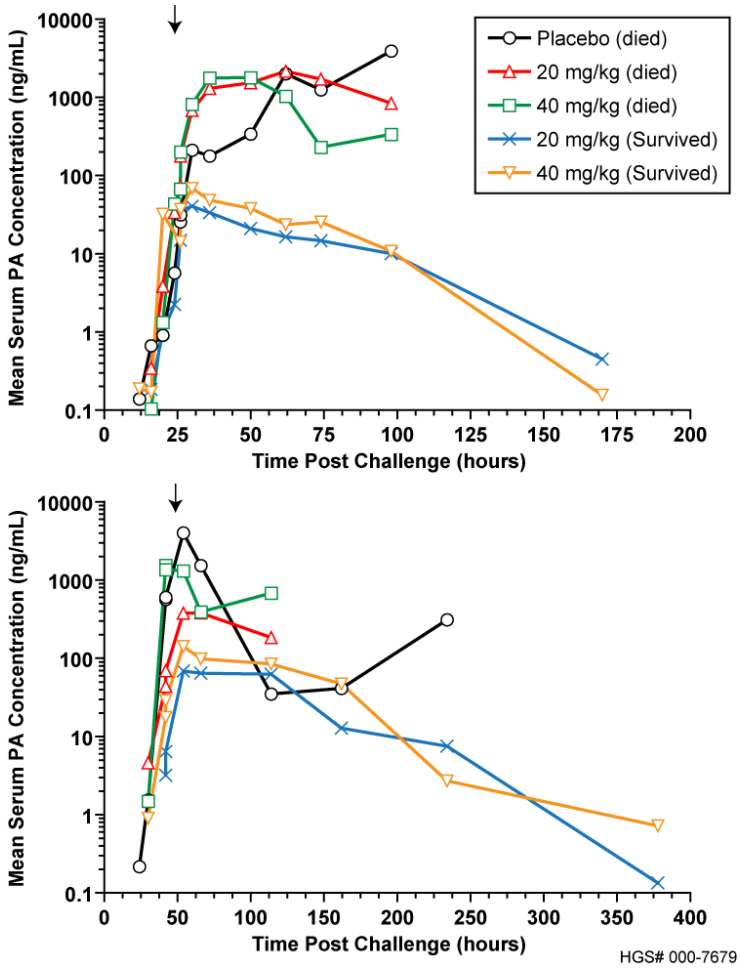

Figure 1-4 Mean serum PA profiles in rabbits (682-G005758) and monkeys (724-G005829)

Upper panel, rabbits; lower panel, monkeys. Arrow indicates median time to administration of raxibacumab or placebo. Note: the decrease in serum PA in the placebo-treated monkeys after 50 hours represents 2 monkeys that lived until Day 10.

Raxibacumab-treated rabbits and monkeys surviving inhalation anthrax develop robust toxin neutralizing activity (TNA). While the levels of TNA are undetectable at baseline, TNA develops in surviving animals by 14 days in some animals and by 28 days in all animals tested, rising to titers well in excess of 400, a level associated with anti-toxin protection. Moreover, anthrax-infected monkeys who were treated with raxibacumab and survived mounted an anti-toxin immune response that was 100% protective against a repeat anthrax spore challenge 1 year later.

Because raxibacumab would be used with to antimicrobials, the activity of antimicrobials were evaluated in combination with raxibacumab. Per agreement with FDA, animal studies were performed in which a full human-equivalent dose of levofloxacin or ciprofloxacin was administered at the same time as raxibacumab to animals with symptomatic disease. Because antimicrobials are most effective when all spores have germinated, administering the

antimicrobials after the animals had become septic maximized the efficacy of the antibiotics and this is reflected in the high survival rates in both the antibiotic alone and raxibacumab/antibiotic combination treatment groups. In contrast, survival rates are highest with raxibacumab when it is given as the PA is first being produced, with 90-100% survival rates in rabbits and monkeys when raxibacumab is administered at the time of spore challenge or at 12 hours after spore challenge. In the clinic when neither the time of spore exposure, onset of symptoms, nor individual time course of disease is easily identified, administering both antimicrobials to kills bacteria and anti-PA antibody to neutralize toxin is a good strategy for combating the source and effects of the disease regardless of its stage of progression.

Per agreement with FDA for an indication as a therapeutic treatment, the safety of raxibacumab has been evaluated in over 400 healthy human volunteers, including 326 subjects treated at the proposed dose of 40 mg/kg with product manufactured by the process proposed for licensure. Raxibacumab was safe and well tolerated and non-immunogenic with single or repeat dosing. Adverse events were generally mild to moderate and did not occur at a rate significantly different from that observed among placebo-treated subjects. A low incidence of mild to moderate rash was observed in some subjects. These rashes were transient and resolved without medication or with oral diphenhydramine. Prophylactic administration of oral diphenhydramine is recommended with raxibacumab treatment. Concomitant administration of raxibacumab with ciprofloxacin did not alter the safety or PK of either antibiotic or raxibacumab.

The mean elimination half-life of raxibacumab was approximately 22 days following a single intravenous (IV) administration and the PK was linear across the 1 to 40 mg/kg IV dose range. In human clinical trials, administration of 40 mg/kg raxibacumab achieved serum levels of raxibacumab that were comparable with those in rabbits and monkeys that provided maximum survival benefit in the efficacy studies with inhalation anthrax.

Having met the requirements of the Animal Rule by demonstrating the efficacy of raxibacumab in rabbit and monkey anthrax inhalation survival studies, the safety and tolerability of raxibacumab in humans, and the ability to translate animal to human drug exposure, 20,000 doses of raxibacumab were accepted into the SNS in 2009 for use by the CDC in the event of an anthrax emergency. This delivery to the SNS fulfilled the contract granted to HGS by BARDA in 2006 for the procurement of new anthrax therapeutics. Subsequent to this initial order, BARDA has contracted with HGS to supply an additional 45,000 doses of raxibacumab to the SNS over the course of the next 3 years.

Unlike antimicrobials which are broadly available to prescribing physicians to treat many different kinds of infections, from mild to severe, caused by a range of organisms, raxibacumab was designed to act specifically with the *B. anthracis* toxin and to be used in the setting of immediately life-threatening inhalation anthrax. Because there are no rapid tests for anthrax bacteria or toxin in humans, initial diagnosis and treatment is based on clinical suspicion of anthrax disease. The current standard of care is to initiate antimicrobial treatment as early as possible to increase the chances of survival. Subjects treated with antimicrobials late in the progression of anthrax infection may be less likely to respond. Initiation of treatment with anti-toxin should have benefit to subjects throughout the course of the illness

because anthrax toxin is central to pathogenesis. As soon as the toxin is present, anti-toxin can protect subjects from its effects. Even in subjects whose bacteremia has been cleared, the toxin can continue to cause significant morbidity and mortality; therefore, anti-toxin therapy should have benefit at any stage of infection.

Raxibacumab is indicated for the treatment of subjects with anthrax infection. Raxibacumab should be used to treat infections that are proven or strongly suspected to be caused by *B. anthracis* bacteria. Raxibacumab provides a significant survival benefit in animals symptomatic for systemic anthrax disease. Earlier treatment is also associated with significant improvement in survival. Raxibacumab is an important treatment option for inhalation anthrax: an effective anti-toxin with a mechanism of action distinct from that of antimicrobials. Raxibacumab neutralizes PA, improves survival, and reduces signs of the disease. It does not prevent the innate immune response and can be used in combination with antibiotics. When used in combination with antibiotics, raxibacumab is not expected to interfere with antibiotic efficacy. Raxibacumab monotherapy is expected to provide clinical benefit for individuals in whom antibiotics are contraindicated or in whom anthrax disease is due to antibiotic-resistant strains of *B. anthracis*.

2 Medical and Public Health Need

Anthrax remains a serious threat to public health. The risk of bioterrorism involving anthrax is real and immediate. Anthrax occurs naturally in soil, regularly causing infections in livestock around the world. Basic microbiology laboratory equipment is sufficient to produce weaponized anthrax. Anthrax spores can be distributed by a variety of means. Once spores are inhaled and germinate, infection progresses rapidly and is usually fatal without prompt treatment. Even with treatment, inhalation anthrax causes significant morbidity and mortality.

Current treatment focuses on antimicrobial therapy. The efficacy of antimicrobials has been demonstrated by in vitro and in vivo research. In a controlled experimental setting, antimicrobials can achieve 100% cure rates against susceptible organisms. However, in real-world clinical use, antimicrobials are not as successful. Experience has shown a survival rate as low as 50% against susceptible strains of anthrax. No one has published research on the effects of multi-drug resistant anthrax, but the expectation is that survival would be considerably lower.

2.1 Clinical Course of Inhalation Anthrax

Anthrax disease is caused by infection with the gram-positive spore-forming bacterium *B. anthracis*. Preparation of *B. anthracis* for inhalation exposure is a highly lethal bioterrorism-related health threat (Holty et al, 2006; Inglesby et al, 2002; Jernigan et al, 2001; Swartz, 2001). The spread of anthrax spores through the US mail system in 2001 prompted the Postal Service to order anthrax testing at over 200 mail centers along the East Coast and random checks nationwide. In the 2001 *B. anthracis* attack in the US, of the 11 patients with inhalational anthrax disease, 5 developed shock (Barakat et al, 2002; Jernigan et al, 2001) and, despite aggressive treatment with antibiotics, fluids and vasopressor support, all 5 died.

Rigorous documentation of the human cases of inhalational anthrax is limited to 2 reports: 42 cases from the Sverdlovsk outbreak in 1979 (Abramova et al, 1993) and the first 10 of 11 cases reported in the US in 2001 (Jernigan et al, 2001). The Sverdlovsk outbreak was notable for the first extensive documentation of necrotizing pneumonia and pathognomonic hemorrhagic mediastinitis. All cases affected previously healthy persons who died after a rapid course of 1 to 4 days duration. Bacteremia was documented in 20 cases and the impact of hematogenous dissemination was characterized by observations of hemorrhagic meningitis (21 cases) and gastrointestinal submucosal hemorrhage (39 cases) (Abramova et al, 1993). The US report focused more on the clinical presentation and confirmed the predominance of abnormal radiologic findings (lung infiltrates, pleural effusions, and mediastinal widening and mediastinal lymphadenopathy) (Jernigan et al, 2001). Of note, extensive pleural effusions requiring drainage were seen in most patients. Laboratory abnormalities included elevation in white cell counts and serum transaminases. Blood cultures were positive in patients who were tested prior to the administration of antibiotics. Cultures rapidly became sterile after initiation of antibiotic therapy indicating that treatment reduces the sensitivity of blood cultures as a diagnostic test.

The deadliest form of the disease, inhalation anthrax, occurs following inhalation of *B. anthracis* spores, which germinate within the macrophages as they travel to the draining mediastinal lymph nodes. Multiplication of the bacteria results in a high organism count in the blood, production of bacterial toxins, and the rapid onset of septicemia. Although the bacterial replication (bacteremia) can be controlled by administration of appropriate antibiotics, it is the bacterial toxin that exerts deleterious effects on the cells within the body, resulting in substantial pathology and high mortality in infected individuals.

The typical clinical course of untreated inhalation anthrax progresses exponentially. The initial asymptomatic phase, with no evidence of germination of the spores, typically lasts 2 to 10 days but has been reported to be as long as 45 days. During this period, treatment with antibiotics has no effect on the spores and actually may prolong this phase by creating an environment unfavorable to germination. Depending on the duration of this phase, there is a chance that the patient may benefit from the administration of anthrax vaccine, but it is unlikely to have a protective effect in time to help the patient.

As germination begins and the anthrax bacteria start replicating there is generally an associated period of 1 to 3 days of prodromal flu-like symptoms. It is at this moment, as the spores germinate, that prompt treatment with antibiotics can have the greatest benefit. If antibiotics are not initiated within 48 hours of the onset of symptoms, the chances of the patient surviving are reduced to 5%.

The transition from a localized infection to systemic illness typically develops rapidly over the course of 12-24 hours. The progression to the intermediate progressive phase is characterized by the onset of systemic manifestations of illness with either bacteremia, pleural effusions, or significant mediastinopathy. Chest radiographs often show pleural effusions and a widened mediastinum, although true pneumonitis is not typically present. Blood cultures are often positive for the characteristic gram-positive spore-forming bacilli. There is rapid clinical deterioration with high fever, dyspnea, and shock. While antimicrobials are essential at this stage for eliminating the bacteria, significant levels of accumulated anthrax toxin can still drive the ongoing progression of disease, resulting in high morbidity and mortality rates.

Progression to the late fulminant stage typically occurs within 6 to 12 hours. The widespread systemic effects of anthrax toxin invariably lead to end organ damage, including hemorrhagic meningitis which present in up to 50% of cases (Cieslak and Eitzen, 1999; Inglesby et al, 2002). The hemodynamic effects of the toxin include severe hypotension with resulting tissue hypoperfusion and hypoxia with profound metabolic acidosis. Large pleural effusions further compound these metabolic and hemodynamic insults that result in substantial morbidity and prolonged need for intensive care. At this phase, there has been no demonstrated benefit from antibiotics.

The 5 fatal cases were patients who presented late during disease progression resulting from a delay in the early initiation of antibiotics. Even among the 6 survivors of the 2001 US anthrax attack who received antibiotics earlier, the duration of hospitalization ranged from 14 to 42 days. The prolonged need for hospitalization despite rapid sterilization of bacteremia reflects the need for therapies that target toxin neutralization and support the likelihood that such an approach will provide substantial clinical benefit.

2.2 Current Treatment Recommendations

2.2.1 Pre-Exposure Prophylaxis

In first responders and military personnel who are at increased risk of exposure to anthrax, vaccination with Anthrax Vaccine Adsorbed (AVA), whose main component is PA, provides adequate protection against anthrax infection. The recommended primary vaccination comprises 2 intramuscular injections at 0, and 4 weeks, and 3 booster vaccinations at 6, 12, and 18 months. After the initial vaccinations, an annual booster shot is recommended to maintain immunity. AVA requires up to 28 days to achieve protective titers of anti-toxin antibody. Once protected, the patient suffers no significant morbidity or mortality from exposure to anthrax. The presence of anti-toxin antibody is protective.

2.2.2 Post-Exposure Prophylaxis

Anyone known or suspected to have been exposed to anthrax, who is not known to have protective immunity, should receive immediate prophylaxis with antibiotics and vaccine injection. The recommended duration of antibiotic is 60 days, and the recommendation is that patients complete the full course of treatment along with the full set of vaccine injections.

2.2.3 Treatment of Symptomatic Disease

CDC guidelines recommend treatment with 2 antimicrobial agents with activity against *B. anthracis*.

2.3 Limitations of Current Treatments

Current treatments are insufficient. The challenge in this rapidly progessing and often fatal disease is the time required to generate an immune response to anthrax toxin. Once patients have generated anti-toxin antibodies, they are protected against anthrax, but the immediate effects of high levels of anthrax toxin are often so rapidly fatal that there is insufficient time for protective immunity to develop. Current treatment is a mixture of antimicrobial therapy and acute supportive care, designed to help the patient survive the infection long enough to mount an immune response. Once patients have the anti-toxin antibody, they are protected against the effects of anthrax. All of the survivors of the 2001 attacks eventually mounted an immune response to the anthrax toxin and developed anti-toxin antibodies by Day 28 after exposure (Quinn et al, 2004).

Because there are no rapid tests for anthrax bacteria or toxin in humans, initial diagnosis and treatment is based on clinical suspicion of anthrax disease. The current standard of care is to initiate antimicrobial treatment as early as possible to increase the chances of survival. Patients treated with antimicrobials late in the progression of anthrax infection may be less likely to respond.

While antibiotics can overcome bacteremia caused by antibiotic-susceptible strains of anthrax, they do not directly address the toxemia that drives pathogenesis. Additional limitations of antibiotics include poor patient compliance with the 60-day schedule and inactivity against antibiotic-resistant strains of *B. anthracis*.

There is a clear need for an anthrax anti-toxin to directly address the toxemia. Anthrax toxin is responsible for the majority of the morbidity and mortality associated with anthrax. Initiation of treatment with anti-toxin should have benefit to patients throughout the course of the illness because anthrax toxin is central to pathogenesis. As soon as the toxin is present, anti-toxin can protect patients from its effects. Even in patients whose bacteremia has been cleared, the toxin can continue to cause significant morbidity and mortality; therefore, anti-toxin therapy should have benefit at any stage of infection.

The treatment paradigm of using anti-toxin in combination with antimicrobials is well established. One example that is very similar is tetanus. In tetanus, the recommended pre-exposure prophylaxis is tetanus toxoid vaccine which induces the production of anti-toxin antibody, conferring protective immunity. Post-exposure prophylaxis includes the use of the vaccine if the patient's immune status is unknown. Treatment of symptomatic infection includes prompt use of antimicrobials in combination with anti-toxin.

3 Development Rationale

The development of raxibacumab arose from the need for an anthrax anti-toxin to fill a gap in the current treatment options. A single IV infusion of raxibacumab delivers recombinant human anti-toxin antibody that is immediately available to block the activity of anthrax toxin. Because of its long half-life, raxibacumab gives patients more time to develop innate immunity through the production of anti-toxin antibody by their own immune system.

3.1 Mechanism of Action of Raxibacumab

B. anthracis produces 3 toxins comprising the binding moiety, PA, and enzymatic moieties, LF and EF (Inglesby et al, 2002). The PA protein binds to its cell surface receptors (CMG2 or TEM8) and is cleaved by a membrane-bound furin-like protease leaving a 63 kDa fragment bound to the cell. This fragment multimerizes into a heptameric barrel structure and exposes a site on PA to which LF and EF bind with high affinity. Internalization of this complex is followed by formation of a membrane-spanning pore. The bound EF and LF proteins are then translocated from the endosome to the cytosol where they exert their toxic effects. EF is an adenylate cyclase which alters cellular homeostasis mechanisms, thereby resulting in edema. LF is a zinc metalloproteinase that induces a hyperinflammatory condition in macrophages resulting in the production of proinflammatory cytokines, contributing to hemodynamic alterations that progress to shock and death of infected subjects.

Raxibacumab inhibits the anthrax toxin effects by preventing PA from binding to its receptor. Raxibacumab binds specifically and with high affinity to PA and inhibits the binding of PA to its receptors. Inhibiting PA binding to its receptors is a key step in protecting cells from the action of anthrax toxin.

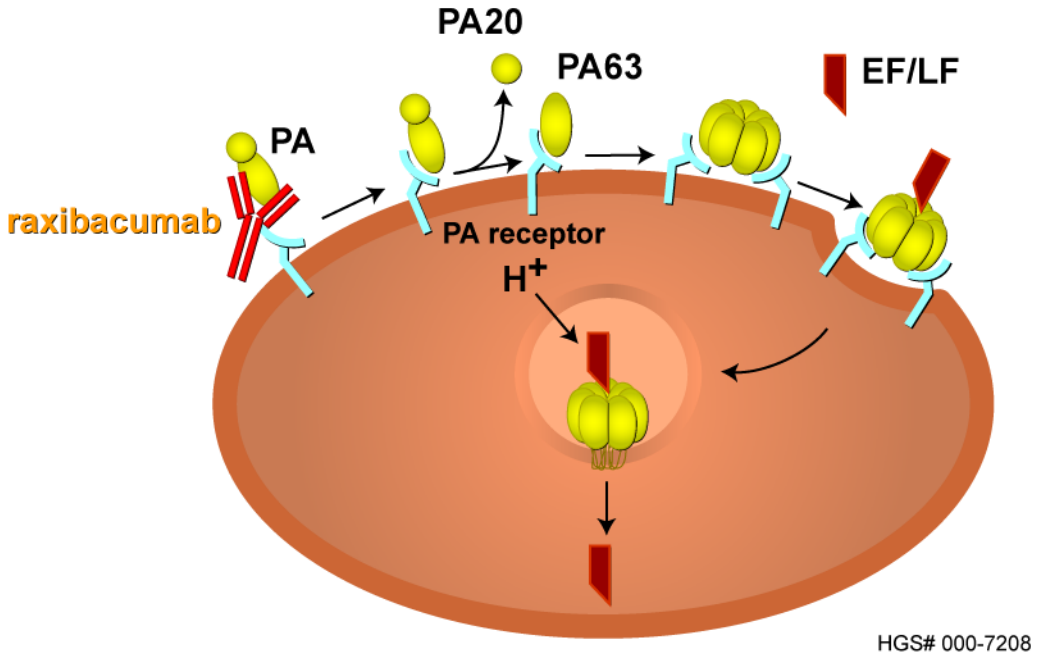

Figure 3-1 Mechanism of action of anthrax toxins and inhibition by raxibacumab

In vitro pharmacology studies have demonstrated that:

- **Raxibacumab binds to PA with high affinity**
  - Raxibacumab showed high affinity binding to PA with an equilibrium binding constant (Kd) of 2.78 nM.

- **Raxibacumab potently inhibits PA binding to anthrax toxin receptor**
  - Raxibacumab inhibition of PA binding is dose dependent with a median inhibitory concentration (IC_{50}) of 503 pM (Figure 3-2).

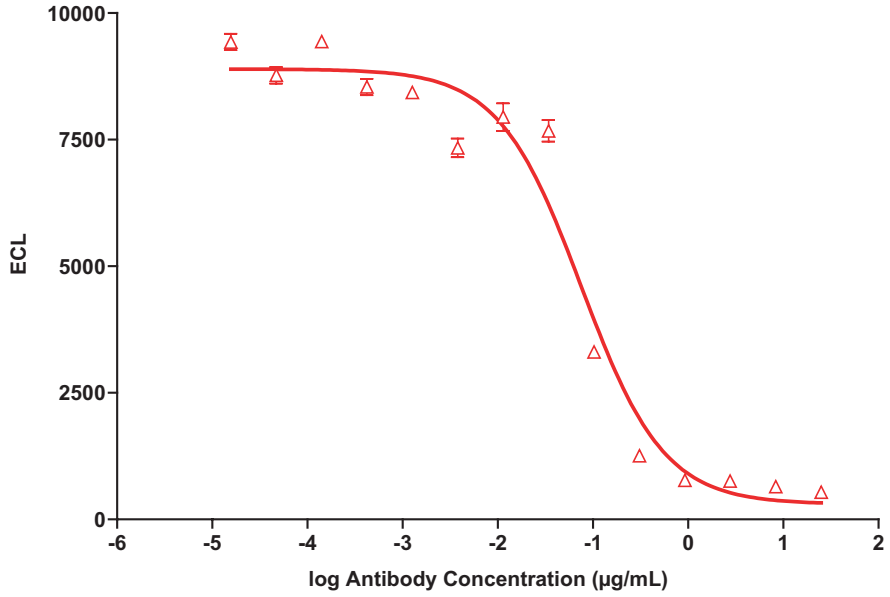

Figure 3-2 Inhibition of PA binding to anthrax toxin receptor with raxibacumab

An ECL-based assay was used to measure the inhibition of PA binding to its recombinant soluble anthrax toxin receptor (TEM8). PA was pre-incubated with raxibacumab before adding receptor. The starting concentration of raxibacumab was 25 µg/mL and a series of 3-fold dilutions were made to 0.0156 µg/mL. Each sample was tested in triplicate; the mean ± SEM is presented

- **Raxibacumab Inhibits the Induction of cAMP by PA/EF**
  - After EF binds to receptor-bound PA it is shuttled into the cell by receptor-mediated endocytosis and internalized, where it causes a rise in cyclic AMP (cAMP) that can be detected by enzyme-linked immunosorbent assay (ELISA). Raxibacumab potently inhibited PA-mediated induction of cAMP by PA/EF with an IC_{50} of 3.5 nM (Figure 3-3).

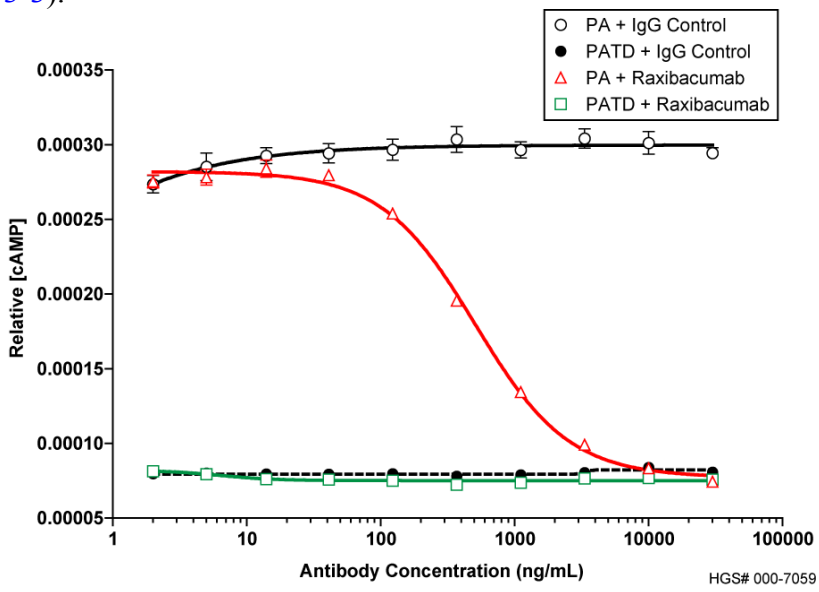

Figure 3-3 Inhibition of cAMP induction by raxibacumab

Raxibacumab or a control IgG_1 antibody were incubated with PA or PATD (defective PA) and EF then added to CHO-K1 cells. Cells were lysed and cAMP induction was detected via chemiluminesence. Using a 4-parameter logistic model, the IC_{50} value was determined to be 509 ng/mL (3.5 nM).

- **Raxibacumab Inhibits Cell Death in Macrophages**
  - Raxibacumab was pre-incubated with PA and added to macrophages, followed by the addition of LF, and cell viability was measured. Raxibacumab inhibited lethal toxin-mediated cell death in a dose-dependent manner with an IC_{50} of 0.21 nM (Figure 3-4). Raxibacumab also inhibited PA-mediated cell death in human macrophages.

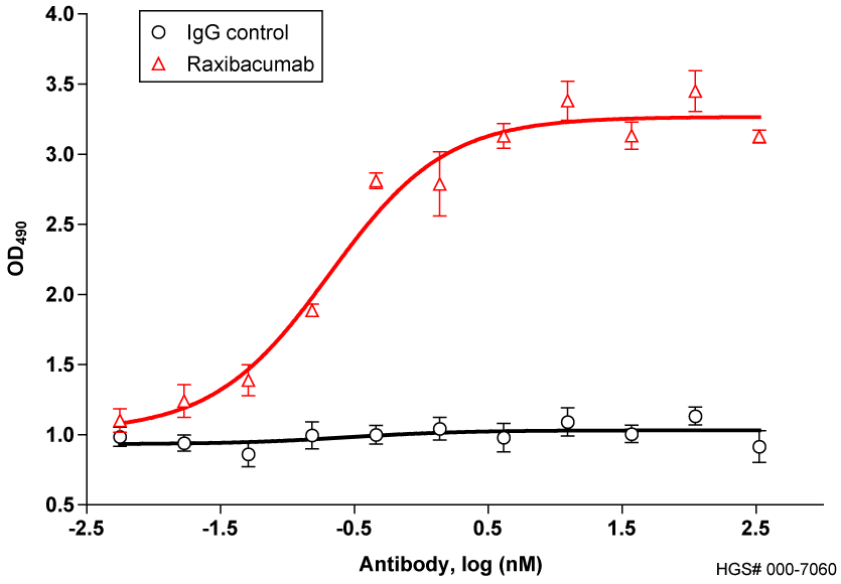

Figure 3-4 Inhibition of lethal toxin-induced cell killing by raxibacumab

Raxibacumab or a control IgG antibody were incubated with PA then added to J774A.1 murine macrophages followed by the addition of LF. Raxibacumab, but not the control antibody, inhibited macrophage killing.

Additional information on the in vitro pharmacology studies is provided in Appendix 13.1.

3.2 PA Epitope which Raxibacumab Binds is Conserved in Different *B. anthracis* Strains

The domains of PA involved in receptor binding have been elucidated (Santelli et al, 2004). Data generated from epitope mapping experiments indicate that the region of PA that is specifically recognized by raxibacumab is involved in the interaction with the receptor. For matters of security, in this document, HGS is not identifying the precise sequence which is recognized, although the exact epitope recognized by raxibacumab has been identified. Raxibacumab recognizes an epitope that has been shown to be highly conserved across the 27 strains of *B. anthracis* that have been sequenced to date. HGS has demonstrated that raxibacumab can bind to 3 strains of *B. anthracis*, Ames, Sterne and Vollum. Given the highly conserved sequence of the region of PA critical for binding to the membrane receptors, raxibacumab should be able to neutralize the toxicity of PA across a broad range of *B. anthracis* strains.

Additional information on the in vitro pharmacology studies is provided in Appendix 13.1.

3.3 Antibiotic-resistant *B. anthracis* Strains

The sequence of 14 antibiotic resistant anthrax strains has been reported. The antibiotic resistance across these strains is due to mutations in one of the following genes:

- Undecaprenyl pyrophosphate phosphatase. This enzyme results in the sequestration of undecaprenyl pyrophosphate. This mutation renders bacteria resistant to bacitracin, a class of cell wall and protein synthesis inhibitors.
- Class A beta-lactamase. This enzyme breaks the beta-lactam antibiotic ring open and deactivates the molecule's antibacterial properites. This mutation renders bacteria resistant to penicillin, a group of cell wall synthesis inhibitors.
- Glutathione transferase, metalloglutathione transferase. This enzyme confers resistance to fosfomycin by catalyzing the addition of glutathione to fosfomycin. Fosfomycin also affects cell wall synthesis.
- rRNA adenine N-6-methyltransferase. This proterin can be methylate adenine at position 2058 of 23S rRNA, conferring resistance to erythromycin, an inhibitor of protein synthesis due to binding to 50S ribosomal subunits.

In these 14 strains, there are no mutations in the region of PA involved in receptor binding, therefore raxibacumab should be active agains these antibiotic-resistant strains.

4 Path to Approval

The proposed indication is:

> Raxibacumab is indicated for the treatment of patients with anthrax infection.
>
> Raxibacumab should be used to treat infection that is proven or strongly suspected to be caused by *B. anthracis* bacteria. When used in combination with antibiotics raxibacumab is not expected to interfere with antibiotic efficacy. Raxibacumab monotherapy is expected to provide clinical benefit for individuals in whom antibiotics are contraindicated or in whom anthrax disease is due to antibiotic-resistant strains of *B. anthracis*.

Because the indication being sought for raxibacumab is the treatment of subjects with anthrax infection, the pivotal efficacy studies have been performed in the therapeutic treatment setting with raxibacumab or placebo administration triggered by clinical signs of systemic anthrax disease. As current standard of care for inhalation anthrax includes use of 2 or more antimicrobials, raxibacumab was also studied in combination with antimicrobials to ensure that raxibacumab does not interfere with their activity.

In addition to the studies demonstrating the survival benefit provided by raxibacumab when used as therapeutic treatment of symptomatic subjects, the efficacy studies performed in the setting of pre-exposure prophylaxis and post-exposure intervention are included as supportive information demonstrating the increased survival rate and time provided by raxibacumab in these settings, the dose-dependence of the raxibacumab survival benefit, and that earlier administration of raxibacumab in the course of anthrax disease is associated with increased survival. This body of data argues for rapid diagnosis of anthrax disease and then immediate intervention with raxibacumab and antibiotics as the best approach to improving survival outcome.

4.1 Animal Rule

Because it is not feasible or ethical to perform controlled clinical trials in which humans are purposely exposed to anthrax, the development program for raxibacumab was designed to meet the criteria described in the "Animal Rule" ("Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible") including demonstration of efficacy in 2 animal species, evaluation of safety in humans, and PK data to translate the exposures in animals to recommended doses in humans. HGS has executed a development program that meets all of the criteria under the Animal Rule for use of animal efficacy data to support the licensure of raxibacumab for therapeutic treatment of inhalation anthrax.

1. Reasonably well-understood pathophysiological mechanism for the toxicity and its amelioration or prevention by the product

As described in Section 3.1, the mechanism of anthrax toxin toxicity is well-understood and the contributions of PA have been elucidated. In vitro nonclinical studies demonstrate that

raxibacumab binds PA with high affinity and specifically interferes with the binding of PA to its receptors thereby preventing the killing of murine and human macrophages by anthrax toxins. These studies were further extended to in vivo models in the rat, rabbit, and cynomolgus monkey, which demonstrate that raxibacumab provides protection from the lethal effects of anthrax toxins and improves survival of infected animals.

2. Demonstration of effectiveness in more than 1 animal species expected to react with a response predictive for humans.

HGS has demonstrated a statistically significant survival benefit over placebo in the pivotal efficacy studies in NZW rabbits (682-G005758) and in cynomolgus monkeys (724-G005829) (Section 8.1). A single IV dose of 20 mg/kg or 40 mg/kg provided a statistically significant and medically meaningful survival benefit when administered as a therapeutic treatment to animals symptomatic for inhalation anthrax as evidenced by detectable serum PA or sustained increased temperature in rabbits and detectable serum PA in monkeys.

In addition, studies were conducted in rabbits (615-N104504) and monkeys (685-G005762) prior to initiation of the pivotal efficacy studies to further characterize the clinical progression of inhalation anthrax disease in each model. These studies added to the body of data available in the literature, confirmed that the pathophysiology of anthrax disease in rabbits and monkeys is similar to that observed in humans, and confirmed that detectable serum PA could be used as a trigger for therapeutic intervention in the animal studies (Section 7.2).

The efficacy of antibiotics administered concomitantly with raxibacumab in the therapeutic treatment setting also was evaluated in NZW rabbits (781-G923701) and cynomolgus monkeys (789-G923702) with symptomatic anthrax disease. In these studies, raxibacumab did not alter the substantial efficacy of levofloxacin or ciprofloxacin administered at doses providing exposures comparable to those achieved in humans administered recommended doses of the antimicrobials (Section 8.2).

3. Animal study endpoint is clearly related to the desired benefit in humans

The primary endpoint, survival rate, is relevant to the outcome of the human disease, which is highly lethal. The secondary endpoint of survival time is also important because longer survival times permit a longer window in which to provide medical intervention to resolve the infection and to allow development of innate immunity against PA.

4. Pharmacokinetics and pharmacodynamics of the product in animals and humans is sufficiently well understood to allow selection of an effective dose in humans

Raxibacumab PK has been evaluated in rabbits, cynomolgus monkeys, and humans (Section 9.1). Raxibacumab has consistent and predictable PK, with comparable peak exposures across the species. For humans administered a 40 mg/kg IV raxibacumab dose, serum raxibacumab levels are rapidly achieved that are comparable to the concentrations that provide a survival benefit in rabbits and monkeys, with immediate high exposure and sufficient concentrations for an adequately prolonged duration. More than 95% of humans administered a 40 mg/kg IV raxibacumab dose can be expected to have serum raxibacumab

concentrations that are equimolar to or in excess of the highest observed serum/plasma PA concentration in any animal that died in the therapeutic efficacy studies (for 48 and 28 days in rabbits and monkeys, respectively). A single 40 mg/kg IV raxibacumab dose in humans is adequate to bind at least 99.7% of serum PA for at least 28 days after administration, in at least 95% of subjects (Section 9.1.7). A 28-day duration of protective raxibacumab concentrations is relevant, since it allows sufficient time for an innate immune response to develop.

All studies subject to the Animal Rule must be conducted in accordance with pre-existing requirements under the Good Laboratory Practice (GLP) regulations and the Animal Welfare Act. The pivotal efficacy studies and the raxibacumab/antibiotic combination efficacy studies to support licensure under the Animal Rule have been conducted in accordance with GLP and the Animal Welfare Act. Human clinical trials which provide the safety data to support the safe use of the product were performed in compliance with Good Clinical Practice (GCP) and International Conference on Harmonisation (ICH) guidelines.

In addition to meeting the criteria under the Animal Rule, HGS addressed the essential elements required for the animals models to be used in studies to support efficacy found in FDA guidance (Animal Models - Essential Elements to Address Efficacy Under the Animal Rule), as described in detail in Appendix 13.8.

4.2 Regulatory History

In response to the anthrax attacks in 2001, the US government issued a mandate under Project Bioshield to make available new countermeasures to fight bioterrorism, which specifically included development of novel therapies to treat inhalation anthrax. A coordinated effort between FDA, which provided the regulatory guidance and framework for licensure; BARDA, which provided resources for development and procurement of raxibacumab; CDC, which manages the SNS and distribution of raxibacumab; Battelle Biomedical Research Center (BBRC), which conducted the inhalation studies in their Biosafety Level (BL3) facilities, and HGS, which executed the nonclinical, clinical, and manufacturing program, resulted in inclusion of raxibacumab in the SNS in January 2009. Raxibacumab licensure remains a goal of HGS as well as a commitment to BARDA.

The following section provides a brief summary of the key regulatory events significant to the development of raxibacumab. A pre-IND meeting was held between HGS and the FDA on 10 October 2002 to discuss the proposed development plans for raxibacumab. The IND application for raxibacumab was subsequently filed to the FDA on 22 May 2003 and included a Phase 1 protocol to evaluate the safety, tolerability, and PK of raxibacumab in healthy volunteers (PAM-NH-01).

HGS filed a request for Fast Track designation which was granted on 15 August 2003 for the investigation of raxibacumab for post-exposure prophylaxis and adjunctive therapy of inhalation anthrax disease, and for lessening the severity of inhalation anthrax disease, including disease caused by antibiotic resistant organisms. A request for Orphan Drug designation for raxibacumab for the treatment of subjects with inhalation anthrax was

submitted to the Office of Orphan Product Development and granted on 12 November 2003 for the treatment of anthrax

During the course of 2004 to 2007, a series of meetings was held for the additional animal efficacy studies required to support licensure and/or use in the SNS. Through these meetings, as well as in follow-up written correspondences from the FDA, the size of the human safety database, the need for repeat dose experience and safety experience of raxibacumab in combination with ciprofloxacin were established. These interactions also established the requirement to demonstrate efficacy in 2 species (rabbits and monkeys); that the animals had to have evidence of systemic anthrax disease at the time of raxibacumab administration for an indication in therapeutic treatment; that serum PA could be used as a trigger for therapeutic treatment; and that the antibiotic exposure in animals in the raxibacumab/antibiotic combination studies should approximate the exposure achieved by the recommended dose in humans. Agreement on the division of studies between those needed to support submission of an IND by the CDC to use raxibacumab in the (SNS) and those additional studies needed for licensure was also achieved.

During 2007, HGS submitted the protocols and analysis plans for the animal efficacy studies, rabbit study 682-G005758 and monkey study 724-G005829. The anthrax inhalation studies were performed at BBRC, West Jefferson, OH, at its BL3 containment facility. The pivotal rabbit and confirmatory monkey efficacy studies with raxibacumab were completed in 2007. The protocols for the raxibacumab/antibiotic combination studies (rabbit study 781-G923701 and monkey study 789-G923702) were completed in the summer of 2008.

A raxibacumab/ciprofloxacin combination safety and PK study in humans (HGS1021-C1064) was conducted in 88 subjects in 2007 and a re-injection study (HGS1021-C1069) enrolling 20 subjects from the HGS1021-C1064 study was performed between January and May 2008. The large human safety study (HGS1021-C1063) enrolling 322 subjects was completed in the summer of 2008.

With completion of the animal and human studies required for licensure in the summer of 2008, a pre-BLA meeting was scheduled and held in October 2008. HGS submitted BLA 125349 electronically to FDA in May 2009 and received notification that the raxibacumab application was granted priority review in July 2009. The results of the pivotal animal studies and human safety trials were published in the New England Journal of Medicine in July 2009 (Migone et al, 2009).

HGS acknowledges and appreciates the time, thought, and effort that the FDA has devoted to the raxibacumab development program. In the setting of the first application for a biologic to be considered under the Animal Rule, the responsiveness, flexibility, and openness of the dialog with the Agency have been invaluable to HGS and to adding this new therapeutic for the treatment of inhalation anthrax to the nation's biodefense capabilities.

5 The Product

Raxibacumab is a recombinant, fully human, $IgG_1\lambda$ monoclonal antibody that binds PA with high affinity and inhibits PA binding to anthrax toxin receptors. Raxibacumab is expressed in mouse myeloma cell line (NS0) cells and the secreted raxibacumab monoclonal antibody is recovered from the cell culture production medium and purified using a series of chromatographic and filtration steps. The clinical product is a single-use liquid product stored at 2-8 degrees Celcius (ºC). Each vial contains 50 mg/mL raxibacumab in 0.13 mg/mL citric acid, 2.8 mg/mL sodium citrate, 10 mg/mL sucrose, 18 mg/mL glycine, and 0.2 mg/mL polysorbate 80, pH 6.5. The vial contains 34 mL (1700 mg) of raxibacumab as a clear to opalescent, colorless to pale yellow solution. The stability of raxibacumab at the recommended storage temperature of 2-8ºC is being monitored in ongoing studies and the data currently support a shelf-life of at least 24 months. Additional studies including excipient robustness and stress studies were performed to confirm that the selected formulation provided adequate stability throughout the intended product shelf-life.

The same raxibacumab formulation has been used for all of the animal safety and efficacy studies and the human safety studies, including the studies that enabled the IND and the studies that support licensure. Raxibacumab is manufactured by HGS. The manufacturing process proposed for licensure is designated the M11 process. Raxibacumab produced by the M11 process has been used in the pivotal rabbit and monkey therapeutic efficacy studies of raxibacumab alone and in combination with antibiotics, and the HGS1021-C1063, HGS1021-C1064, and HGS1021-C1069 human clinical trials. In the BLA, HGS submitted the data for 59 lots of bulk drug substance and 14 lots of final product, along with process validation and product characterization data that demonstrate the consistency and robustness of the manufacturing process. The product intended for commercialization is the same as that used in the principal studies supporting licensure.

6 Nonclinical Safety

The nonclinical safety program was designed to support single and repeat-dosing in clinical trials with healthy volunteers. The target for raxibacumab is a bacterial toxin and should not be present in healthy animals and humans. Ex vivo tissue cross-reactivity studies were conducted in rabbit, monkey, and human tissues to support the nonclinical safety program and confirmed the limited reactivity of raxibacumab with human and animal tissues. A repeat-dose toxicology study conducted in cynomolgus monkeys and an embryo-fetal study performed in NZW rabbits demonstrated that raxibacumab was safe and well-tolerated. The nonclinical safety program for raxibacumab was conducted in consideration of the recommendations given in the ICH and FDA guidelines.

6.1 Choice of Species

The target for raxibacumab is a bacterial toxin, PA. Theoretically, this target should not be present in any healthy species used for nonclinical safety testing, and therefore, the choice of relevant species was based upon the relevant species selected for the determination of efficacy of raxibacumab in inhalation anthrax exposure studies. The efficacy studies used the NZW rabbit and the cynomolgus monkey, as these are considered pathophysiologically-relevant to the human disease. The cynomolgus monkey was chosen for safety assessment because of its phylogenetic proximity to humans, and because the monkey may be less likely to develop immunogenicity to a fully human monoclonal antibody with repeated injection than would a rabbit. Since immunogenicity can negatively impact PK and therefore drug exposure, the monkey was chosen as the relevant species for initial toxicity studies.

6.1.1 GLP Tissue Cross-Reactivity Study with Raxibacumab in Rabbit, Monkey and Human Tissues

Two ex vivo tissue cross-reactivity studies were conducted with raxibacumab to identify potential targets of raxibacumab binding in rabbit, monkey, and human tissues and to correlate whether binding was apparent in these same tissues in animals chosen as a relevant nonclinical toxicology species.

In the 1st study, raxibacumab was evaluated in cynomolgus monkey and human tissues using product manufactured by a development process (M10). In the 2nd study, product from the M11 manufacturing process proposed for licensure was evaluated in rabbit, cynomolgus monkey, and human tissues. Consistent with the target of raxibacumab not being an endogenous protein, there was little cross-reactivity observed in human, monkey, or rabbit tissues. Variably frequent thyroid binding was observed in humans and monkeys in both of the tissue cross reactivity studies. However, none of the ex vivo tissue binding observations translated into adverse events or altered chemistry or laboratory findings in the animal toxicology study or human clinical studies. A detailed description of the tissue cross-reactivity studies is provided in Appendix 13.4.

6.1.2 Repeat-dose Toxicology Study in Cynomolgus Monkeys

A GLP repeat-dose toxicity study was conducted which included 18 cynomolgus monkeys (9/gender), approximately 2 to 5 kilograms in weight. Raxibacumab was administered by either subcutaneous (SC) or IV injection (Group 2 and Group 3, respectively) at a dose volume of 1 mL/kg on Days 1, 13, and 25. Group 1 monkeys received an equal dose volume of raxibacumab diluent as the vehicle control divided between the SC and IV routes (0.5 mL/kg delivered by each route). Dose frequency was based on the half-life of raxibacumab in cynomolgus monkeys (approximately 12-17 days) from a single dose PK study conducted previously.

On Study Day 50, monkeys were to be returned to a non-naïve colony. On Day 69, all monkeys were returned to the study because the study design was amended to include both a long-term immunogenicity sample collection for the SC monkeys (after Study Day 100), and the inclusion of IM dosing and subsequent histopathology of limited tissues, based on the results of the GLP tissue cross-reactivity study. In addition, on Day 69, monkeys in Group 1 and Group 3 were administered a single IM injection of diluent or raxibacumab (40 mg/kg, divided into 2 sites, 0.4 mL/kg/site), respectively, in the thigh. Monkeys in Group 2 were left untreated but evaluated for clinical observations, body weights, food consumption, and immunogenicity/neutralization antibodies. On Day 77, monkeys in Group 1 and Group 3 were necropsied, gross observations recorded, and tissues were collected for hisological examination.

In-life observations included twice daily mortality/moribundity evaluations and cageside observations, daily qualitative food consumption, and weekly body weight and detailed clinical observations. Blood samples were routinely taken for hematology, serum chemistry, complement activation (C3a), and immunogenicity evaluations. To confirm proof of exposure, samples were collected at or near predicted C_{max} for each route.

The following conclusions can be drawn based upon the results of the repeat-dose toxicology study in cynomolgus monkeys. Raxibacumab, administered either SC, IV, or IM, was well tolerated in cynomolgus monkeys. There were no changes in clinical observations, body weights, or food consumption. There were no alterations in the clinical pathology data that could be attributed to the SC, IV, or IM administration of raxibacumab. IV administration of raxibacumab was associated with mild decreases in erythrocyte number and hematocrit in males, an effect that may be related to treatment or to multiple bleeding intervals, or some contribution of both. There did not appear to be activation of complement with repeated raxibacumab administration to cynomolgus monkeys. There were no gross observations or histomorphological changes suggestive of a treatment effect in monkeys sacrificed after IM dosing. Repeat dosing of raxibacumab was not immunogenic.

Additional information on the repeat-dose toxicology study is provided in Appendix 13.6.

6.1.3 Embryo-Fetal Toxicology Study in Rabbits

A GLP embryo-fetal development and toxicokinetics study was conducted in which 69 female NZW rabbits, between 5 and 6 months old, were mated to males of the same strain and assigned to treatment groups receiving raxibacumab by IV injection at a dose of 0, 40, or

120 mg/kg on Gestational Days 7 and 14. The 40 mg/kg raxibacumab dose reflected the intended dose in human safety studies and the 120 mg/kg raxibacumab dose was selected to reveal dose related trends and capture any harmful effect associated with raxibacumab. Dose timing and frequency was based on the period of organogenesis in NZW rabbits. The IV route of injection was selected because it is the intended route of administration in humans.

All rabbits survived to the scheduled termination with no remarkable treatment-related clinical signs noted for any group and there were no treatment-related maternal necropsy findings. The pregnancy rate was 95% for the control and 120 mg/kg groups and 100% for the 40 mg/kg group. There were no abortions or early deliveries. All pregnant rabbits had a litter with viable fetuses. The mean number of corpora lutea, implantation sites, mean percent preimplantation and postimplantation loss, and mean number of live fetuses were similar across all groups, which indicated that treatment with raxibacumab had no effect on embryo or fetal viability.

There were no fetal external variations. Malrotated hindlimbs were noted in one 40 mg/kg fetus. This was not considered treatment-related due to the absence of a dose-response relationship. The total fetal soft tissue variations were similar across all groups. The types noted in this study are commonly seen in this strain of rabbit and all of the fetal and litter incidences were within the historical control range at this laboratory. The malformation of persistent truncus arteriosus was noted in one 40 mg/kg fetus but was not considered treatment-related due to the absence of a dose-response relationship and an incidence that was within the historical control range.

The total fetal skeletal variations and malformations were similar across all groups. For the skeletal parameters evaluated, fetal variations and malformations were of the type and frequency commonly seen in this strain of rabbit and were all generally within the historical control range. The fetal incidences of 5^{th} sternebra unossified and 13^{th} rudimentary ribs were significantly increased for the 120 mg/kg dose group. However, these findings were not attributed to raxibacumab since the litter incidence was similar to control, the findings were within the historical control range, and there was an absence of a dose-response relationship in the incidences of total fetal and litter skeletal variations.

Incidence of anti-raxibacumab responses was low (0, 2, and 2 responses following a single IV dose in placebo, 40 mg/kg, and 120 mg/kg raxibacumab treated rabbits, respectively). Although the presence of anti-raxibacumab antibodies may affect raxibacumab PK, raxibacumab exposure (albeit attenuated) was maintained throughout the study for the anti-raxibacumab antibody positive rabbits.

The conclusions of the study were that the no-observable-adverse-effect level (NOAEL) for raxibacumab when administered by IV injection on Gestational Day 7 and 14 to pregnant rabbits during the period of organogenesis is 120 mg/kg for maternal toxicity, the highest dose tested on study and the no-observable-effect level (NOEL) for embryo/fetal viability, growth, and fetal development (teratogenicity) is also 120 mg/kg/dose.

Additional information on the embryo-fetal toxicology study is provided in Appendix 13.7.

7 Animal Models

Rabbit and non-human primate models of inhalation anthrax are well established and have been used to evaluate vaccines and to support licensure of antibiotics. Prior to the initiation of the pivotal efficacy studies with raxibacumab, studies were conducted in rabbits and monkeys to further characterize the clinical progression of inhalation anthrax disease in each model. These studies confirmed that inhalation anthrax in rabbits, monkeys, and humans share a common pathophysiology and natural history. The organ involvement and types of inflammatory and hemorrhagic lesions are similar. Development of systemic bacteremia is contemporaneous with the detection of PA in the blood, which established detectable PA as a trigger for intervention of symptomatic animals. In the therapeutic treatment models the endpoint is improved survival, the desired outcome in humans. A detailed discussion of the rabbit and monkey inhalation models and how they address the FDA guidance regarding essential elements required for design of animal efficacy studies (Animal Models - Essential Elements to Address Efficacy Under the Animal Rule) is provided in Appendix 13.8.

7.1 Rabbits and Non-human Primate Models of Inhalation Anthrax are Well Established

The pathology of the inhalational form of the disease in non-human primates is remarkably similar to that observed in human (Fritz et al, 1995; Twenhafel et al, 2007; Vasconcelos et al, 2003;). The most significant pathology is edema, hemorrhage, and necrosis with leukocyte infiltration in various tissues including mesenteric and tracheobronchial lymph nodes, meninges, lungs, and small intestine (Fritz et al, 1995). However, splenic pathology was different between humans and non-human primates, since all of the non-human primate studies demonstrated splenomegaly. In addition, the severity of the neutrophilic inflammation and the fibrin exudation appears to be greater in the spleens of non-human primates. Mediastinal enlargement due to different degrees of edema and hemorrhage is a consistent finding across chimpanzees, rhesus macaques, and cynomolgus monkeys (Vasconcelos et al, 2003). Henderson and his colleagues provided data suggesting that inhaled *B. anthracis* spores in the lung alveoli of rhesus macaques initiate infection and are phagocytosed by macrophages which either remain in the primary infection site or migrate to regional lymph nodes (Henderson et al, 1956).

After an inhalation of a lethal dose of *B. anthracis* spores, death occurs in non-human primates typically between 2 and 10 days with a median of approximately 4 days. The LD_{50} in rhesus macaques has been shown to be 5.5×10^4 spores, while it is 4.13×10^3 spores in the cynomolgus macaque (Vasconcelos et al, 2003). Clinical signs of the infection include fever, lethargy, weakness, and anorexia manifested generally 1 to 4 days preceding death. Bacteremia is also present for 2 days before death. Over the past decades, extensive research on non-human primates for inhalational anthrax has made the non-human primate model a well-suited system to evaluate new therapies for inhalational anthrax. The non-human primate model was used to establish the efficacy of ciprofloxacin, doxycycline, and levofloxacin in post-exposure prophylaxis setting (Friedlander et al, 1993; Kao et al, 2006 Meyerhoff et al, 2004).

Several rabbit species have been used as models to study inhalational anthrax infection; the most widely employed being the NZW rabbit. Lesions observed in NZW rabbits were comparable with those of inhalational anthrax in humans and rhesus monkeys (Zaucha et al, 1998). The most significant pathological changes occurred in the lymph nodes, spleen, lungs, adrenal glands, and gastrointestinal tracts. Notable differences are lack of leukocyte infiltration in brain and meningeal lesions, the relatively mild mediastinal lesions, and a lower incidence of anthrax-related pneumonia compared with humans. This discrepancy may be attributed to the rapid progression of disease in this species, which presumably, limits development of leukocyte infiltrates in response to hemorrhage and necrosis (Zaucha et al, 1998).

The mean survival time of rabbits with inhalational anthrax is 2.4 days, and clinical signs are not manifested until within 24 hours of death. The calculated LD_{50} for *B. anthracis* Ames strain in rabbits is 1.1 x 10^5 colony forming units (CFU) which is higher than the LD_{50} in non-human primates. Although the rapid fatal course of inhalation anthrax could be considered disadvantageous, the rabbit model has several attractive features compared with the well-developed rhesus macaque model: rabbits are considered lower phylogenic species and are easier to obtain and safer to house and handle than non-human primates (Phipps et al, 2004).

7.2 Model Characterization Studies Establish PA as Trigger for Intervention

Per recommendation by FDA, to support an indication in therapeutic treatment, raxibacumab was to be administered to animals that were symptomatic for anthrax disease. Model characterization studies were conducted in NZW rabbits and cynomolgus monkeys to identify clinical or laboratory parameters that could be used as triggers for therapeutic intervention. These studies are summarized below; additional discussion of the studies that identified the triggers for intervention is provided in Appendix 13.9.

Animals were challenged with a target challenge of 200 x LD_{50} of *B. anthracis* spores (Ames strain). Study 615-N104504 included 8 naïve rabbits (4 male and 4 female) and Study 685-G005762 included 8 monkeys (7 male and 1 female) who had survived a monkey pox study. Parameters measured included clinical observations, temperature, hematology, CRP, serum PA, serum raxibacumab, and bacteremia by culture and PCR. Rabbits were monitored for 7 days; monkey for 28 days. TNA was also measured in the surviving animals.

The primary analysis of the studies was examination of the relationship between survival time and time to onset of clinical parameters indicative of anthrax infection including bacteremia (by culture and PCR), detectable serum PA, and clinically significant increase in body temperature.

The findings from the model characterization studies include:

7.2.1 Survival Rate and Survival Time

- 7/8 died rabbits within the 7-day post-exposure monitoring period; the 8th animal was euthanized at the end of the pre-specified 7-day monitoring period. 6/8 monkeys died during the 28-day monitoring period.
- Earliest death in rabbits was at 48 hours; latest death was greater than 168 hours for the animal that was euthanized at study end. Earliest time to death in monkeys was 85 hours; latest death was 156 hours.
- No statistically significant correlation between the extent of spore exposure and the time to death in either species was observed.

7.2.2 Bacteremia and Toxemia

- All rabbits and all monkeys became toxemic or bacteremic by culture during the study.
- Earliest observed bacteremia by culture in rabbits was at 20 hours after spore challenge. The median time to bacteremia was 26 hours. Bacteremia measured by PCR in rabbits was consistent with earliest observed bacteremia at 20 hours and median time to bacteria of 28 hours. Bacteremia by culture or PCR in monkeys was observed as early as 30 hours post challenge and the median time to bacteremia was 36 and 39 hours, respectively
- Earliest detectable PA in rabbits was 24 hours (the earliest time tested) and the median time to detectable PA was 30 hours. Earliest detectable PA in monkeys was 30 hours and median time to detection was 39 hours.
- The mean serum PA concentration-time profiles in non-survivors in both species show an initial rise in serum PA up to about 36 hours post challenge, followed by a period during which the concentrations plateau, followed by a 2nd period of rising concentrations.
- For the animals that died, there were no significant correlations between serum PA kinetic parameters and the magnitude of the spore challenge.
- Appearance of detectable PA was coincident with appearance of bacteremia by culture or PCR.

7.2.3 Temperature Increase

- All rabbits had clinically significant temperature increases (ie, ≥ 2 degrees Fahrenheit [°F]) after spore challenge. The time to clinically significant temperature increase ranged from 26 to 78 hours with a median time to temperature increase of 32 hours.
- All 8 monkeys demonstrated a diurnal temperature pattern with body temperatures elevated during the day and reduced at night. In addition, consecutive temperature readings tended to be erratic. 5/8 animals showed a dramatic decrease in body temperature as they became moribund. In the 2 survivors, temperature cycling returned to normal between 14 to 21 days post challenge.

7.2.4 Hematology and CRP

- In rabbits and monkeys, white blood cells (WBC) were the most indicative of the hematology parameters for disease progression. WBC was relatively stable in all of the spore-challenged rabbits through 20 hours post spore challenge. From 24 to 36 hours, most rabbits exhibited sharp declines in WBC. These decreases were followed over the

next 2 days by increases in WBC to levels at or exceeding baseline values. In the anthrax-exposed monkeys, WBCs were relatively stable in all of the animals through 24 hours post spore challenge. For 2 animals, increases in WBC above the normal range occurred before PA and bacteremia were detected. For all other animals, increases in WBC were observed at or after the appearance of detectable PA and/or bacteremia. This pattern of WBC is consistent with the initial recruitment of WBC to the site of infection followed by the increase in circulating WBC.

- CRP was measured throughout the study as an indicator of systemic inflammation. Elevations in CRP were observed in most of the rabbits and the increases in CRP generally trailed the onset of bacteremia and temperature increase. Elevations in CRP were observed in all of the monkeys and the increases in CRP were generally delayed compared with the onset of toxemia and bacteremia.

7.2.5 Time to Event for Clinical Parameters

- Serum PA and bacteremia by culture or PCR are the 1st indicators of anthrax disease in rabbits and monkeys, followed shortly thereafter by temperature rise and changes in WBC, and subsequently by increases in CRP. The appearance of PA in serum is coincident with the appearance of bacteremia, and is an antecedent to increased temperature. This sequence of events is observed in both rabbits (Figure 7-1) and monkeys (Figure 7-2).

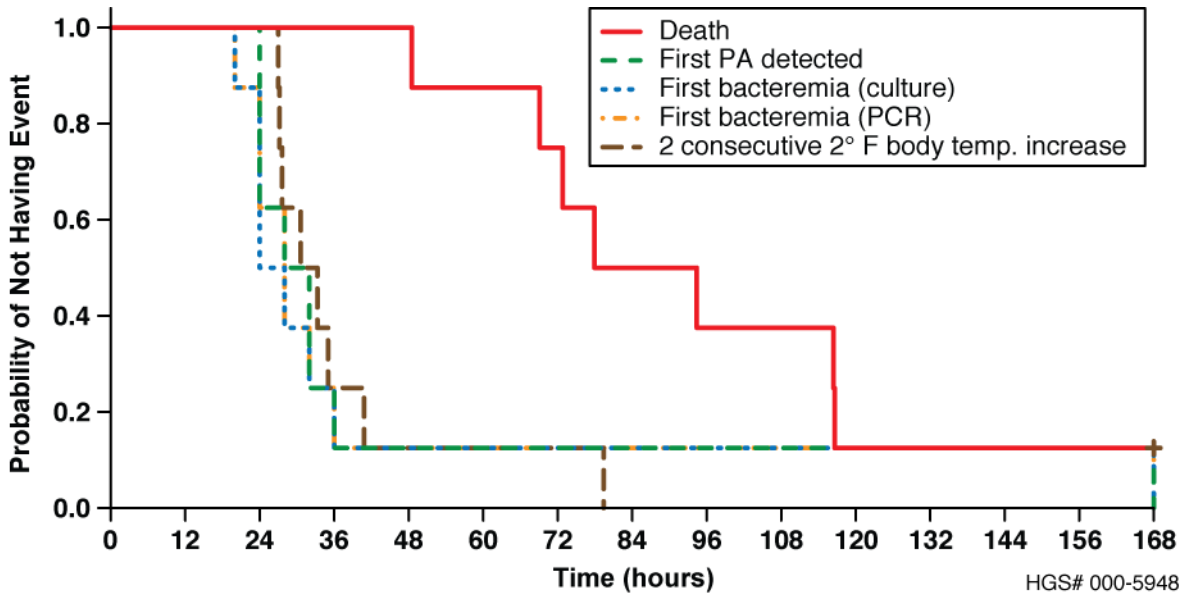

Figure 7-1 Time to event for clinical parameters in rabbits (Study 615-N104504)

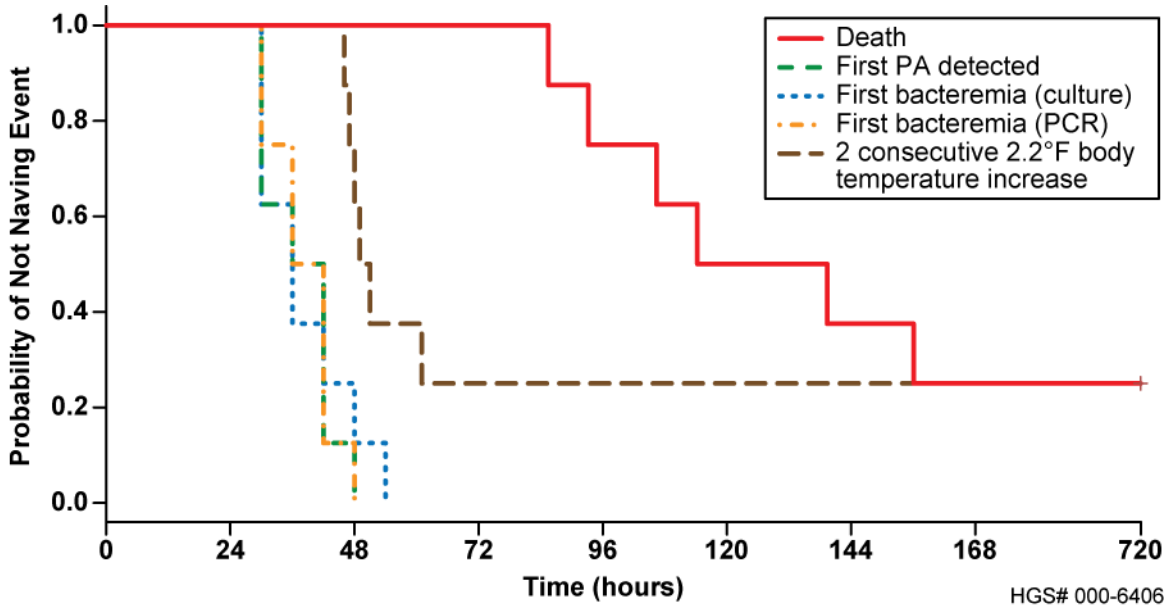

Figure 7-2 Time to event for clinical parameters in monkeys (Study 685-G005762)

- Survival time was strongly correlated with the time to detection of PA (r = 0.896, p = 0.0026 for rabbits and r = 0.93, p = 0.0008 for monkeys) indicating that the appearance of serum PA was a good predictor of mortality.
- Detection of bacteremia measured by culture was consistent with bacteremia detection by PCR, and survival time was strongly correlated with time to bacteremia by culture (r = 0.854, p = 0.0070 for rabbits; r = 0.96, p = 0.0001 for monkeys) and PCR (r = 0.952, p = 0.0003 for rabbits; r = 0.85, p = 0.0082 for monkeys).
- Survival time was also strongly correlated with time to a significant increase in body temperature (r = 0.833, p = 0.0102) in rabbits, although less so than with serum PA or bacteremia. Survival time was not correlated with time to a significant increase in body temperature (r = -0.30, p = 0.4685 for 1st significant temperature rise for consecutive temperature elevations) in monkeys.

7.2.6 Toxin Neutralizing Activity

- TNA was measured in samples drawn pre-challenge and at Days 14, 21, and 30 for monkeys that survived to Day 14 or greater. All animals had values below the limit of detection prior to challenge. Only 2 animals were alive at the Day 14 to have samples taken and TNA was measurable in both animals. At Days 21 and 30, TNA titers were > 5000, indicating that both of the surviving animals had mounted a strong immune response to PA. Titers > 400 are considered protective against anthrax toxin.

7.2.7 Choice of Trigger for Therapeutic Intervention

- Serum PA which can be tested in approximately 2 hours was identified as a sensitive and rapid trigger for treatment, as opposed to bacteremia by PCR, which takes hours, or bacteremia by culture, which can take days, to obtain results.
- In the therapeutic efficacy studies in rabbits, serum PA or temperature, whichever occurred first, was used as the trigger for therapeutic intervention. For studies in monkeys, the treatment trigger was serum PA.
- In all of the therapeutic treatment efficacy studies, the presence of bacteremia was confirmed retrospectively by measuring serum bacteria by culture and serum PA at the time of treatment.

8 Efficacy

Raxibacumab has been demonstrated to be efficacious when administered to animals with symptomatic systemic inhalation anthrax in 2 species. Raxibacumab administered as monotherapy neutralizes toxin and significantly improves survival rate, increases survival time, and reduces the signs of infection. Improved survival rate is the desired outcome for anthrax therapies and increased survival time is also a benefit as it provides more time for intervention, particularly important in the human treatment setting, and for innate immunity to develop.

Measurement of innate anti-toxin immunity that develops in surviving animals demonstrated that raxibacumab does not interfere with the natural immune response to anthrax exposure. Rechallenge of raxibacumab-treated anthrax survivors confirms that their innate immunity is robust and protective against anthrax rechallenge.

Raxibacumab administered in combination with antimicrobials was also evaluated in anthrax-infected rabbits and monkeys using levofloxacin and ciprofloxacin, respectively. In both studies, survival rates were high for antimicrobial-treated animals and animals receiving the combination. Based on the distinct mechanism of action of the anti-toxin compared with antimicrobials, it is unlikely that raxibacumab interferes with the efficacy of antibiotics.

At the beginning of the raxibacumab development program, a number of studies were performed examining the efficacy of raxibacumab administered as pre-exposure prophylaxis and post-expsoure intervention. These studies, also conducted in NZW rabbits and cynomolgus monkeys, provide additional information on the dose-dependence of raxibacumab efficacy and the relationship of survival rate to the duration of the time from spore exposure to treatment.

Table 13-7 lists the efficacy studies described in this document in support of the therapeutic treatment indication with species, age, weight, and anthrax spore exposure. Table 13-8 lists the same information for ancillary efficacy studies in pre-exposure prophylaxis and post-exposure intervention. Table 13-9 summarizes the study design, trigger for intervention, the percentage of animals bacteremic or toxemic at the time of treatment with study agent, and the percentage of survivors in the control groups for the therapeutic efficacy studies. Table 13-10 summarizes the same information for ancillary efficacy studies in pre-exposure prophylaxis and post-exposure intervention.

8.1 Survival Benefit Demonstrated in Pivotal Studies in 2 Species

In both rabbits and monkeys, raxibacumab provided a statistically significant improvement in overall survival as well as in time to death and both trials were positive in their prespecified primary endpoint analyses. Moreover, the increased survival benefit afforded by raxibacumab was robust in that the improved survival observed across the prespecified subgroups by bacteremia and/or toxemia status at the time of treatment was consistent with the effect observed in the overall population. Additional details of the pivotal animal efficacy studies are provided in Appendix 13.10.

8.1.1 Study Design

Because the animal anthrax inhalation studies provide the primary efficacy support for licensure, they were designed and performed with the approach used for human clinical trials. The trials were placebo-controlled, randomized, parallel-group comparison. The rabbit study was open-label; the monkey study was double-blind. The studies were adequately powered to provide a statistically significant result if a clinically important increase in survival was observed. Protocols and analytical plans prespecified the study design and data analysis and were submitted to FDA prior to study initiation and appropriate statistical adjustments were made for multiple comparisons of the primary endpoint for the 2 raxibacumab doses.

The designs for the studies were similar and are illustrated in Figure 8-1. The rabbit study (682-G005758) assigned 54 animals to one of 3 treatment groups (18 rabbits/group for 2 raxibacumab groups and 1 placebo group, 50% males and 50% females). The monkey study (724-G005829) assigned 40 cynomolgus monkeys to 2 separate groups of 14 monkeys (raxibacumab) and 1 group of 12 monkeys (placebo) (50% male, 50% female in each group). In both studies, animals were challenged with a target dose of 200 x LD_{50} of anthrax spores (Ames strain).

The trigger for treatment of individual rabbits with raxibacumab or placebo was detectable serum PA or 1st rise in body temperature of 2°F or more above the baseline average at 2 consecutive timepoints (whichever occurred first). For individual monkeys, upon detection of serum PA, a single 1 mg/kg IM dose of diphenhydramine was to be administered, followed within 5 minutes by a single bolus IV injection of either 40 mg/kg or 20 mg/kg raxibacumab, or placebo. Premedication with diphenhydramine was to reflect its use in the human clinical trials. The primary efficacy endpoint was survival on Day 14 in rabbits and on Day 28 in monkeys. Parameters measured included clinical observations, temperature, hematology, CRP, serum PA, bacteremia, and serum raxibacumab in both species; and anti-PA and TNA in the monkeys.

There were no dropouts in any of the efficacy studies and all animals entered on study were followed through death, euthanasia, or the specified follow-up periods for survivors. In Study 682-G005758, 1 rabbit in the placebo group died due to a blood clot resulting from implantation of the venous access port prior to spore challenge, resulting in 17 rather than 18 rabbits in the placebo group. One rabbit in the 20 mg/kg raxibacumab treatment group died of a broken back during the anthrax spore challenge and did not receive study agent. This animal was included as a death in the intention-to-treat (ITT) population in the primary efficacy analysis.

Additional details of the pivotal animal efficacy studies are provided in Appendix 13.10.

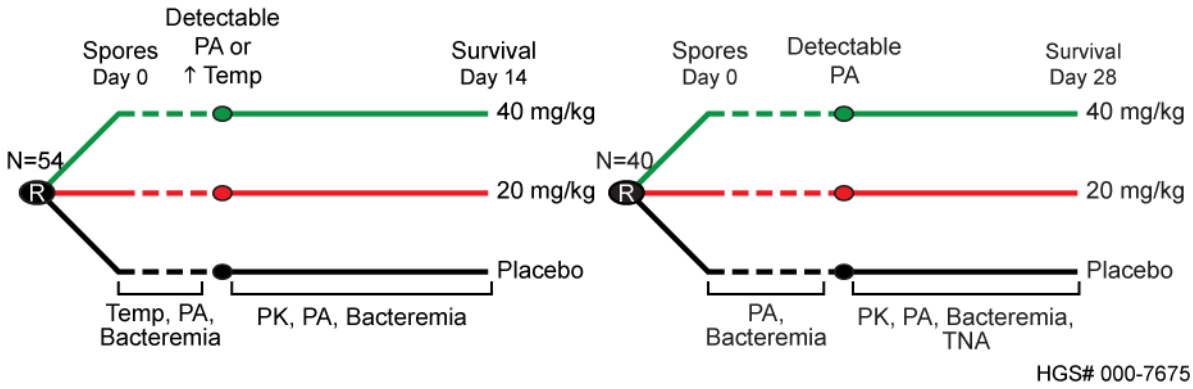

Figure 8-1 **Study design for rabbit (left) and monkey (right) pivotal efficacy studies (682-G005758 and 724-G005829)**

8.1.2 Efficacy Results

The results of the primary endpoint analysis in the pivotal rabbit and monkey studies are shown in Figure 8-2. Both studies met the conditions for a positive trial with statistically significant improvement in survival, including adjustments for multiple comparisons of the 2 active dose groups with placebo. The percent survival for rabbits treated with 20 or 40 mg/kg raxibacumab at the onset of symptoms was 27.8% ($p = 0.0455$) and 44.4% ($p = 0.0029$), respectively, compared with 0% survival in placebo-treated rabbits at Day 14. Similarly, the percent survival for monkeys treated with 20 or 40 mg/kg raxibacumab at the onset of symptoms was 50% ($p = 0.0064$) and 64.3% ($p = 0.0007$), respectively, compared with 0% survival in the placebo-treated monkeys at Day 28. In both studies, the survival rate was numerically higher in the 40 mg/kg raxibacumab dose group than the 20 mg/kg raxibacumab dose group (160% and 129%, relative benefit of 40 mg/kg raxibacumab compared with 20 mg/kg raxibacumab), but these differences did not reach statistical significance.

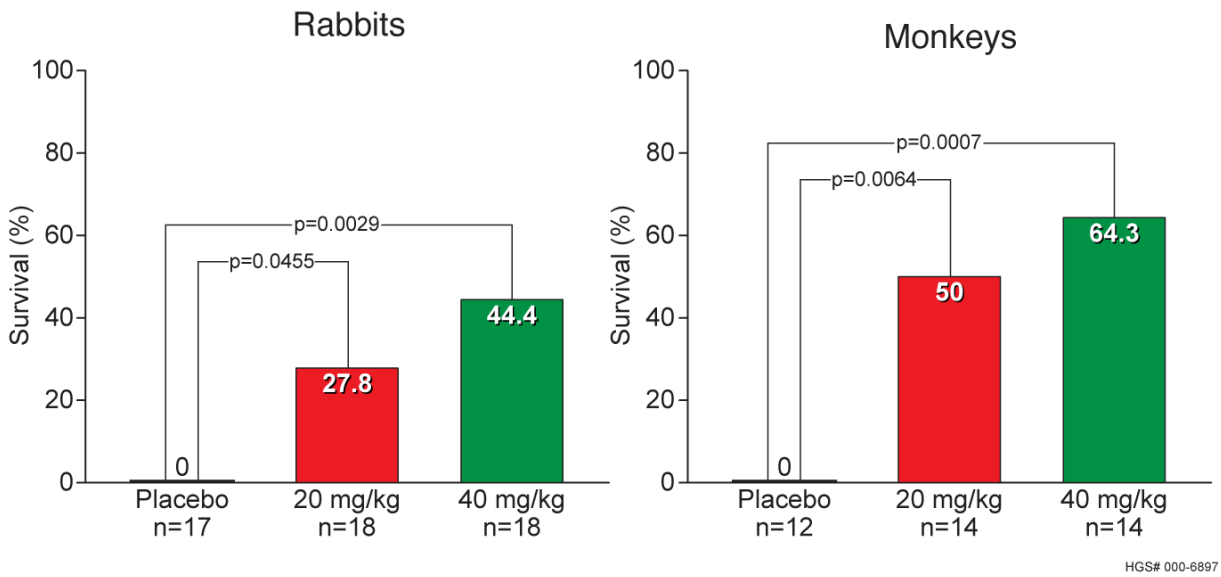

Figure 8-2 **Primary endpoint in the pivotal efficacy study in rabbits (682-G005758) on left and confirmatory efficacy study in monkeys (724-G005829) on right**

8.1.2.1 Sensitivity Analyses of the Primary Endpoint

Sensitivity analyses were performed on the primary endpoint. HGS examined the as-treated population in the rabbit study; the ITT population and as-treated population were the same in the monkey study. HGS performed a sensitivity analysis in the monkey study, excluding 3 animals from the 40 mg/kg raxibacumab group who had lower LD_{50} spore challenges than the placebo-treated animals. FDA performed an analysis in the as-treated population for animals that were confirmed to be bacteremic at the time of treatment for both studies. These analyses are provided in Table 8-1 for the rabbit study and Table 8-2 for the monkey study. In all of the sensitivity analyses, the 40 mg/kg treatment group had a statistically significant higher survival rate than the placebo-treated animals, even with adjustment for multiple comparisons.

Table 8-1 **Sensitivity analyses of primary endpoint in rabbit study (682-G005758)**

Analysis	Population	Treatment	N	Number (%) of Survivors	P-Value[1,2]
HGS	As-randomized[3]	Placebo	18	0 (0.0%)	-
		20 mg/kg raxibacumab	18	5 (27.8%)	0.0455
		40 mg/kg raxibacumab	18	8 (44.4%)	0.0029
HGS	As-treated[4]	Placebo	16	0 (0.0%)	-
		20 mg/kg raxibacumab	17	5 (29.4%)	0.0445
		40 mg/kg raxibacumab	19	8 (42.1%)	0.0038

Table 8-1 Sensitivity analyses of primary endpoint in rabbit study (682-G005758)

Analysis	Population	Treatment	N	Number (%) of Survivors	P-Value[1,2]
FDA	As-treated[4] and bacteremic at the time of treatment	Placebo	13	0 (0.0%)	-
		20 mg/kg raxibacumab	16	4 (25.0%)	0.1067
		40 mg/kg raxibacumab	17	6 (35.3%)	0.0237

1 Two-sided Fisher's exact test for comparison between active treatment and placebo.

2 A positive result required a p-value < 0.05 for both groups or < 0.025 in either group according to the Hochberg adjustment used for the multiple comparisons.

3 One rabbit in the placebo group died prior to spore challenge and is included in the placebo group in the as-randomized analysis.

4 One rabbit in the placebo group inadvertently received 40 mg/kg and is included in the 40 mg/kg group in the as-treated analysis.

(concluded)

Table 8-2 Sensitivity analyses of primary endpoint in monkey study (724-G005829)

Analysis	Population	Treatment	N	Number(%) of Survivors	P-Value[1,2]
HGS	ITT excluding 3 animals with lowest LD_{50}[3]	Placebo	12	0 (0.0%)	-
		20 mg/kg raxibacumab	14	7 (50.0%)	0.0064
		40 mg/kg raxibacumab	11	8 (72.7%)	0.0003
FDA	Bacteremic at the time of treatment	Placebo	10	0 (0.0%)	-
		20 mg/kg raxibacumab	12	5 (41.7%)	0.0396
		40 mg/kg raxibacumab	13	9 (69.2%)	0.0016

1 Two-sided Fisher's exact test for comparison between active treatment and placebo.

2 A positive result required a p-value < 0.05 for the 40 mg/kg raxibacumab group in order to test for superiority in the 20 mg/kg raxibacumab group at p < 0.05 according to the step-down adjustment used for the multiple comparisons.

3 Three monkeys in the 40 mg/kg raxibacumab group had spore challenges less than the lowest challenges received in the placebo group. This analysis excludes these animals with lower LD_{50} exposures.

As further confirmation of the efficacy of raxibacumab in animals with documented anthrax disease, the effect of raxibacumab on survival was evaluated in several subgroups of rabbits and monkeys by their toxemia and bacteremia status at the time of raxibacumab administration. In all of the therapeutic treatment efficacy studies, the increased survival rates observed in various subgroups by bacteremia and/or toxemia status at the time of treatment are consistent with the survival benefit observed in the overall population as shown in Figure 8-3.

Figure 8-3 displays the absolute improvement in survival rate (point estimate and 95% confidence intervals) of the 20 and 40 mg/kg treatment groups compared with placebo for the

prespecified subgroups of the ITT population in Studies 682-G005758 and 724-G005829. The primary efficacy analysis in the ITT population is displayed at the top of the figure and the results in the prespecified subgroups are displayed below it. The dashed vertical line represents the point estimate for the treatment effect in the ITT population. The solid vertical line indicates 0% added benefit. Consequently, point estimates to the right of the solid vertical line (shown as an oval) indicate a higher survival rate compared with placebo. Horizontal bars that cross the ITT point estimate (dashed vertical line) convey that the effect in these subgroups is included within the effect in the overall population.

In Figure 8-3, for all of the prespecified subgroups by toxemia, bacteremia, or increased temperature status at the time of study agent administration, the point estimate of the survival benefit is contained within the confidence intervals of the main effect. This demonstrates that the survival benefit in the prespecified subgroups is consistent with the effect observed in the overall ITT population and that the survival benefit was maintained in the animals that were confirmed to be bacteremic and or toxemic at the time of treatment.

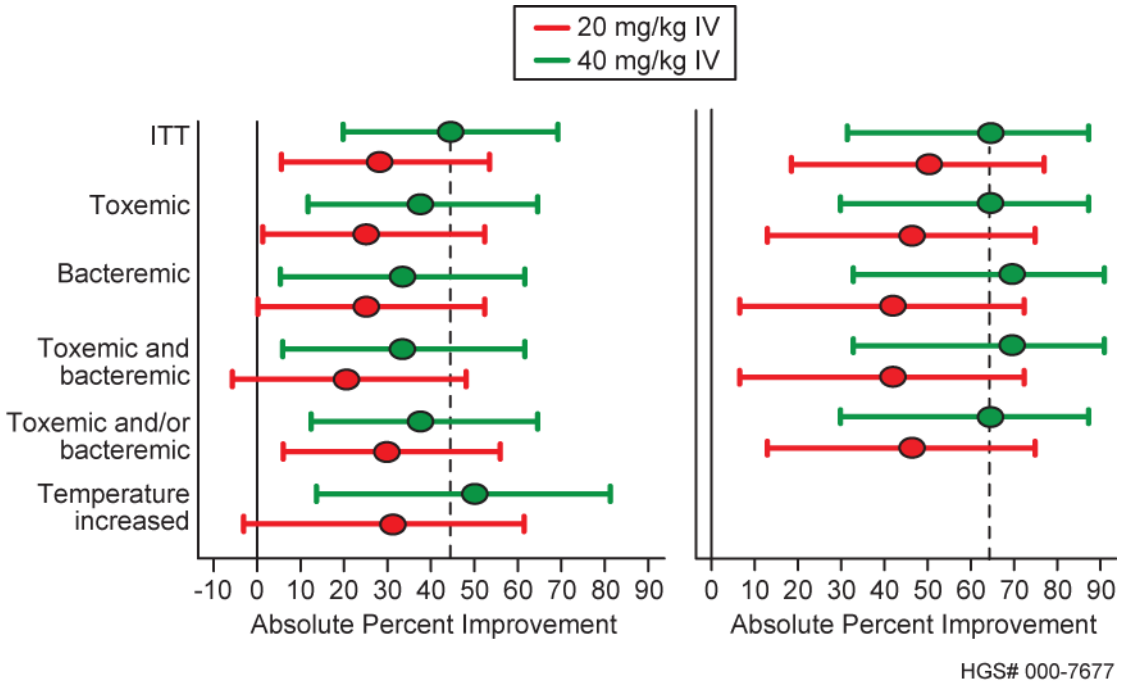

Figure 8-3 **Absolute improvement and unconditional exact 95% CI in survival compared with placebo by subgroup of the ITT population (rabbit study 682-G005758, left; monkey study 724-G005829, right)**

8.1.2.2 Increased Survival Time

The secondary efficacy endpoint was survival time defined as the time from spore challenge to death during the index study interval in the ITT population. For animals that were alive at the end of the index study interval, survival times were censored on the date of study completion.

In the pivotal therapeutic efficacy studies in both species, 20 mg/kg and 40 mg/kg raxibacumab significantly increased survival time compared with placebo and the trend to increased survival time was dose-dependent (Figure 8-4).

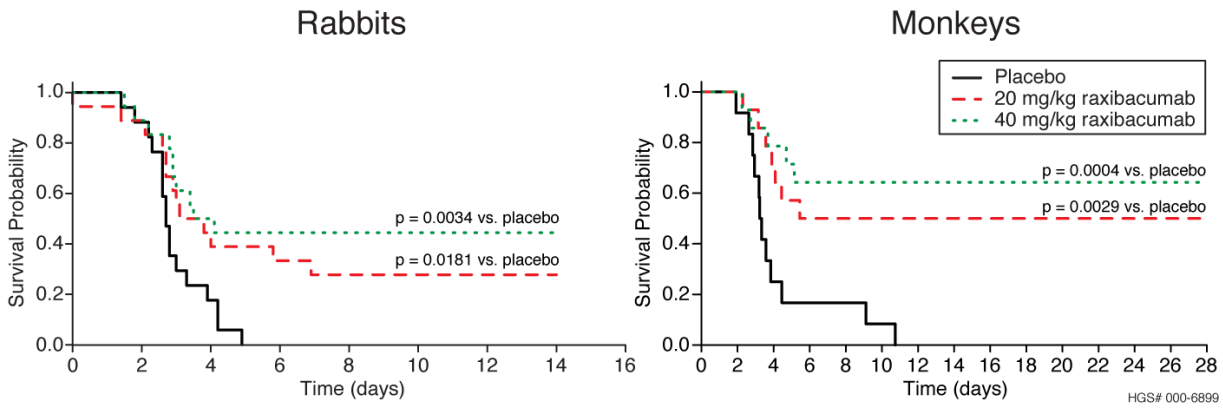

Figure 8-4 **Survival time in rabbits (682-G005758) on left and monkeys (724-G005829) on right**

The statistically significant survival benefit provided by raxibacumab in rabbits and monkeys in adequately powered trials with prespecified primary endpoints addresses the requirements under the Animal Rule for demonstration of effectiveness in more than 1 animal species expected to react with a response predictive for humans with a primary endpoint of improved survival rate, which is the desired benefit in humans.

8.2 Raxibacumab Does Not Interfere with the Action of Antibiotics

Currently the treatment for inhalation anthrax includes post-exposure prophylaxis with antimicrobials. Because raxibacumab is likely to be administered in combination with antimicrobials in the event of acute exposure to anthrax, HGS performed efficacy studies in spore-challenged rabbits and monkeys with raxibacumab in combination with levofloxacin and ciprofloxacin, respectively. Concomitant administration of raxibacumab and antibiotics results in a statistically significant increase in survival rate and survival time and reduces the signs of anthrax disease. Raxibacumab does not interfere with efficacy of the antibiotics.

8.2.1 Study Design

Human-equivalent doses of levofloxacin and ciprofloxacin, respectively, were administered with 40 mg/kg raxibacumab to rabbits and monkeys with systemic anthrax disease. The

designs for the double-blinded, randomized placebo-controlled studies were similar and are illustrated in Figure 8-1. The rabbit study, (781-G923701) assigned 52 animals to one of 3 treatment groups (20 rabbits/group for each of the 2 active treatment groups and 12 rabbits in the placebo group, 50% males and 50% females). The monkey study (789-G923702) assigned 40 cynomolgus monkeys to 2 separate groups of 14 monkeys each (active treatment) and 1 group of 12 monkeys (placebo) (50% male, 50% female in each group). In both studies, animals were challenged with a target dose of 200 x LD_{50} of anthrax spores (Ames strain).

The trigger for treatment of individual rabbits was detectable serum PA or 1st rise in body temperature of 2°F or more above the baseline average at 2 consecutive timepoints (whichever occurred first); the trigger for intervention in the monkeys was detectable serum PA.

In the rabbit study, 50 mg/kg levofloxacin was administered once daily by oral gavage for 3 days with or without concomitant administration of a single IV dose of 40 mg/kg raxibacumab; the control group received placebo. In the monkey study, 75 mg ciprofloxacin was administered twice daily by oral gavage for 3 days with or without concomitant administration of a single IV dose of 40 mg/kg raxibacumab; the control group received placebo. Monkeys also received a single 1 mg/kg IM dose of diphenhydramine administered 5 minutes before administration of study agent to reflect the use of diphenhydramine in the human clinical trials. Per discussion with FDA, the antibiotic regimen chosen for the raxibacumab/antibiotic combination studies was to have been adequate to demonstrate sterilization of bacteremia in the animals and to represent a human equivalent dose of the antimicrobial. In both studies, bacteria were sterilized by the antimicrobials within 3 days, and generally within 24 hours.

Primary efficacy endpoint was survival on Day 28 in rabbits and monkeys. Parameters measured included clinical observations, temperature, hematology, serum PA, bacteremia, serum raxibacumab, TNA, and mean inhibitory concentration (MIC) in both species. Gross necropsy and histopathology were performed on all non-surviving animals and the surviving rabbits were also sacrificed for histologic examination. The surviving monkeys were not sacrificed.

Additional information on these studies is provided in Appendix 13.11.

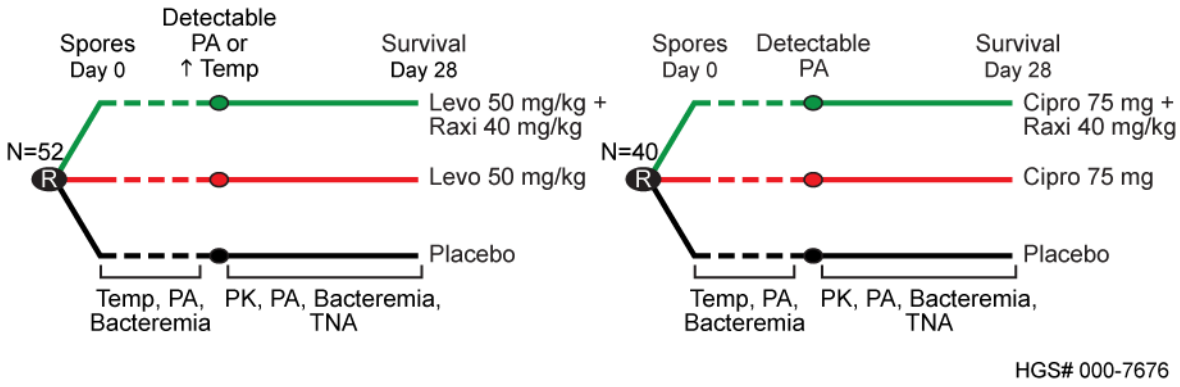

Figure 8-5 **Study design for rabbit (left) and monkey (right) raxibacumab/antibiotic combination efficacy studies (781-G923701 and 789-G923702)**

8.2.2 Efficacy Results

In both studies, the primary efficacy endpoint in the ITT population was met, with statistically higher 28-day survival in the antibiotic/raxibacumab groups and the antibiotic groups (both 19/20 (95.0%) with levofloxacin/raxibacumab and levofloxacin in Study 781-G923701, and 12/14 (87.5%) for ciprofloxacin/raxibacumab and 14/14 (100%) for ciprofloxacin in Study 789-G923702; $p < 0.0001$ for all active treatment groups compared with 0% survival in the placebo-treated animals as shown in Figure 8-6. There was no difference in survival rates between the 2 active treatment groups ($p = 1.0000$ based on Fisher's exact test) in either study.

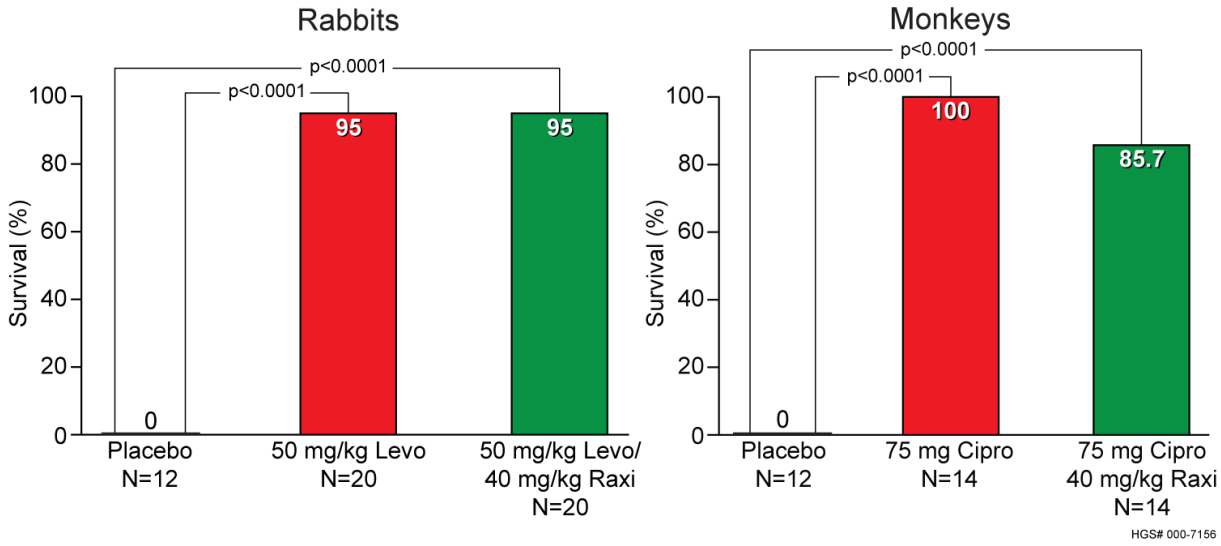

Figure 8-6 **Primary endpoint in raxibacumab/antimicrobial combination efficacy studies with levofloxacin in rabbits (781-G923701) on left and with ciprofloxacin in monkeys (789-G923702) on right**

8.2.2.1 Sensitivity Analyses of the Primary Endpoint

Sensitivity analyses were performed on the primary endpoint. HGS examined the as-treated population in the rabbit study because 1 rabbit in the raxibacumab/levofloxacin treatment group was determined by PK results not to have received raxibacumab. In addition, 1 rabbit in the levofloxcin treatment group died of a gavage error, and not from anthrax. HGS performed a sensitivity analysis on the as-treated population excluding this animal. In the monkey study, 1 monkey in the raxibacumab/ciprofloxacin treatment group died as the result of a gavage error, not of anthrax and HGS performed a sensitivity analysis of the primary endpoint excluding this animal. In the monkey study, the ITT population was the same as the as-treated population. The FDA performed sensitivity analyses on the as-treated population in rabbits which were bacteremic at the time of treatment, and performed a sensitivity analysis with the population of monkeys demonstrated to be bacteremic at the time of treatment. FDA also performed an analysis of rabbits and monkeys which were confirmed to be toxemic at the time of treatment using the randomized, rather than as-treated, population.

The results of these analyses are provided in Table 8-3 for the rabbit study and Table 8-4 for the monkey study. In all of the sensitivity analyses, the raxibacumab/antibiotic treatment group had a statistically significant higher survival rate than the placebo-treated animals, even with adjustment for multiple comparisons using the Hochberg method. In none of the studies was the survival rate significantly different in the antibiotic alone and raxibacumab/antibiotic treatment groups.

Using antibiotic doses that produced equivalent exposure to that achieved by the recommended human doses as required by FDA, produced survival rates in the antibiotic-only groups too high to enable demonstration of added benefit of raxibacumab.

Table 8-3 Sensitivity analyses of primary endpoint in rabbit raxibacumab/levofloxacin combination study (781-G923701)

Analysis	Population	Treatment	N	Number(%) of Survivors	P-Value[1,2]
HGS	As-treated[3]	Placebo	12	0 (0.0%)	-
		Levofloxacin	21	19 (95.4%)	< 0.0001
		Raxibacumab/levofloxacin	19	18 (94.7%)	< 0.0001
HGS	As-treated[3] excluding the animal that died of a gavage error	Placebo	12	0 (0.0%)	-
		Levofloxacin	21	19 (95.4%)	< 0.0001
		Raxibacumab/levofloxacin	18	18 (100%)	< 0.0001
FDA	As-treated[3] and bacteremic at the time of treatment	Placebo	10	0 (0.0%)	-
		Levofloxacin	20	19 (95.0%)	< 0.0001
		Raxibacumab/levofloxacin	17	16 (94.1%)	< 0.0001
FDA	As-treated[3]	Placebo	12	0 (0.0%)	-
		Levofloxacin	21	19 (95.4%)	< 0.0001
		Raxibacumab/levofloxacin	19	18 (94.7%)	< 0.0001

Table 8-3 Sensitivity analyses of primary endpoint in rabbit raxibacumab/levofloxacin combination study (781-G923701)

Analysis	Population	Treatment	N	Number(%) of Survivors	P-Value[1,2]
FDA	As-randomized and toxemic at the time of treatment	Placebo	12	0 (0.0%)	-
		Levofloxacin	19	18 (94.7%)	< 0.0001
		Raxibacumab/levofloxacin	18	17 (94.1%)	< 0.0001

1 Two-sided Fisher's exact test for comparison between active treatment and placebo.

2 A positive result required a p-value < 0.05 for both groups or < 0.025 in either group according to the Hochberg adjustment used for the multiple comparisons.

3 One rabbit in the raxibacumab/levofloxacin group did not receive raxibacumab and is include in the levofloxacin group in the as-randomized analysis.

(concluded)

Table 8-4 Sensitivity analyses of primary endpoint in monkey raxibacumab/ciprofloxacin combination study (789-G923702)

Analysis	Population	Treatment	N	Number(%) of Survivors	P-Value[1,2]
HGS	ITT, excluding the animal that died of a gavage error	Placebo	12	0 (0.0%)	-
		Ciprofloxacin	14	14 (100%)	< 0.0001
		Raxibacumab/ciprofloxacin	13	12 (92.3%)	< 0.0001
FDA	As-treated[3] and bacteremic at the time of treatment	Placebo	10	0 (0.0%)	-
		Ciprofloxacin	13	13 (100%)	< 0.0001
		Raxibacumab/ciprofloxacin	13	11 (84.6%)	< 0.0001
FDA	As-treated[3]	Placebo	12	0 (0.0%)	-
		Ciprofloxacin	14	14 (100%)	< 0.0001
		Raxibacumab/ciprofloxacin	14	12 (85.7%)	< 0.0001
FDA	As-randomized and toxemic at the time of treatment	Placebo	11	0 (0.0%)	-
		Ciprofloxacin	13	13 (100%)	< 0.0001
		Raxibacumab/ciprofloxacin	14	12 (85.7%)	< 0.0001

1 Two-sided Fisher's exact test for comparison between active treatment and placebo.

2 A positive result required a p-value < 0.05 for both groups or < 0.025 in either group according to the Hochberg adjustment used for the multiple comparisons.

3 All animals received the treatment to which they were randomized, so the ITT population is the same as the as-treated population.

As further confirmation of the efficacy of raxibacumab in combination with antibiotics, survival rates were evaluated in several subgroups of rabbits and monkeys by their toxemia and bacteremia status at the time of raxibacumab administration. The survival benefit in the prespecified subgroups was consistent with the effect observed in the overall ITT population and the survival benefit was maintained in the animals that were confirmed to be bacteremic and or toxemic at the time of treatment.

8.2.2.2 Increased Survival Time

In the raxibacumab/antibiotic combination studies, levofloxacin or ciprofloxacin alone and in combination with raxibacumab significantly increased survival time compared with placebo (Figure 8-7). In Study 781-G923701, the animal in the levofloxacin/raxibacumab group that died from the gavage error rather than anthrax disease died early at 2 days after challenge, while the animal that died of anthrax succumbed later at 10 days. Similarly, in Study 789-G923702, the animal that died from the gavage error died early at 3.7 days and the animal that died of anthrax disease died late at 10 days.

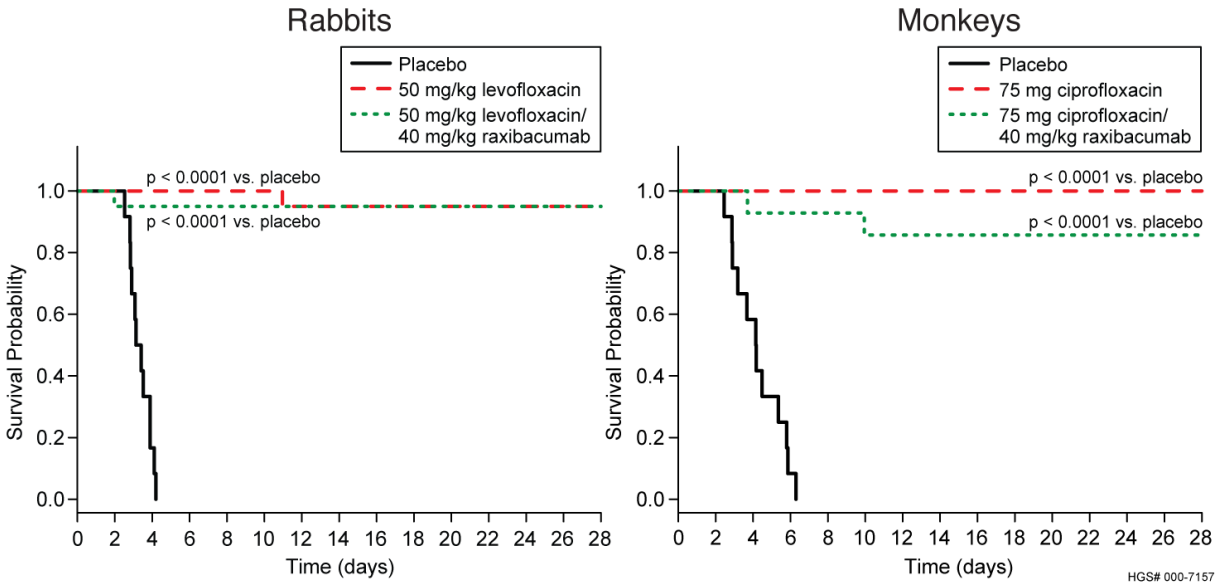

Figure 8-7 **Survival time in raxibacumab/antimicrobial combination studies with levofloxacin in rabbits (781-G923701) on left and with ciprofloxacin in monkeys (789-G923702) on right**

8.3 Dose and Time-dependence Demonstrated in Pre-Exposure Prophylaxis and Post-Exposure Intervention Studies

The initial raxibacumab efficacy studies were performed in the setting of pre-exposure prophylaxis and post-exposure intervention to evaluate the effects of dose and timing relative to spore challenge on survival rate. These studies in rabbits and monkeys demonstrated that the survival benefit is dose-dependent with highest survival afforded by the 40 mg/kg dose. Survival rate was also time-dependent with higher survival with earlier treatment after spore exposure. The statistically significant benefits in survival rate and survival time were demonstrated in both species.

8.3.1 Study Design

Rabbit Study 288-HGSIRAB was a placebo-controlled, randomized, parallel group study in which 72 NZW rabbits were randomized by gender and body weight into each of 6 treatment

groups (12 rabbits/group, 50% males and 50% females) and challenged with a targeted 100 x LD_{50} dose of *B. anthracis* spores (Ames strain). For each rabbit, a single SC administration of placebo or 1, 5, 10, or 20 mg/kg raxibacumab was administered 2 days prior to spore challenge or an IV administration of 40 mg/kg (route due to injection volume constraints) was administered within 1 hour of spore challenge. The time of spore challenge was selected to provide maximum raxibacumab concentrations in the blood at the time of spore challenge. Parameters measured included clinical observations, temperature, hematology, CRP, serum PA, serum raxibacumab, and bacteremia. A complete necropsy was performed on any rabbit that died during the 14-day observation period.

Monkey Study 290-N005433 was a placebo-controlled, randomized, parallel group study in which 40 cynomolgus monkeys were randomized by gender and body weight into each of 4 treatment groups (10 rabbits/group, 50% males and 50% females) and challenged with a targeted 100 x LD_{50} dose of *B. anthracis* spores (Ames strain). For each monkey, a single SC administration of placebo or 10, 20, or 40 mg/kg raxibacumab was administered 2 days prior to spore challenge to provide maximum raxibacumab concentrations in the blood at the time of spore challenge. Parameters measured included clinical observations, temperature, hematology, CRP, serum PA, serum raxibacumab, and bacteremia. A complete necropsy was performed on any monkey that died during the 28-day observation period.

Rabbit Study 358-N005999 was a placebo-controlled, randomized, parallel-group study in which 60 NZW rabbits were randomized by gender and body weight into each of 5 treatment groups (12 rabbits/group, 50% males and 50% females) and challenged with a targeted 100 x LD_{50} dose of *B. anthracis* spores (Ames strain). Each rabbit was administered a single IV dose of 40 mg/kg raxibacumab at 0, 12, 24, and 36 hours after spore challenge; placebo was administered at the time of spore challenge. Parameters measured included clinical observations, temperature, and bacteremia.

Additional information on these studies is provided in Appendix 13.12.

8.3.2 Efficacy Results

8.3.2.1 Raxibacumab Survival Benefit is Dose Dependent

In both rabbits and monkeys, raxibacumab provided a dose-dependent increase in survival when administered at a time to produce maximal serum concentrations at the time of anthrax spore challenge (ie, either Day -2 for SC administration or within 1 hour of spore challenge for IV administration). At doses of 5 mg/kg raxibacumab and above in rabbits and of 10 mg/kg (the lowest dose tested) and above in monkeys, a single dose of raxibacumab provided a statistically significant improvement in survival compared with placebo treatment as shown in Figure 8-8. In both species, doses of 10, 20, and 40 mg/kg produced highly significant improvements in survival with $p < 0.0001$ compared with placebo.

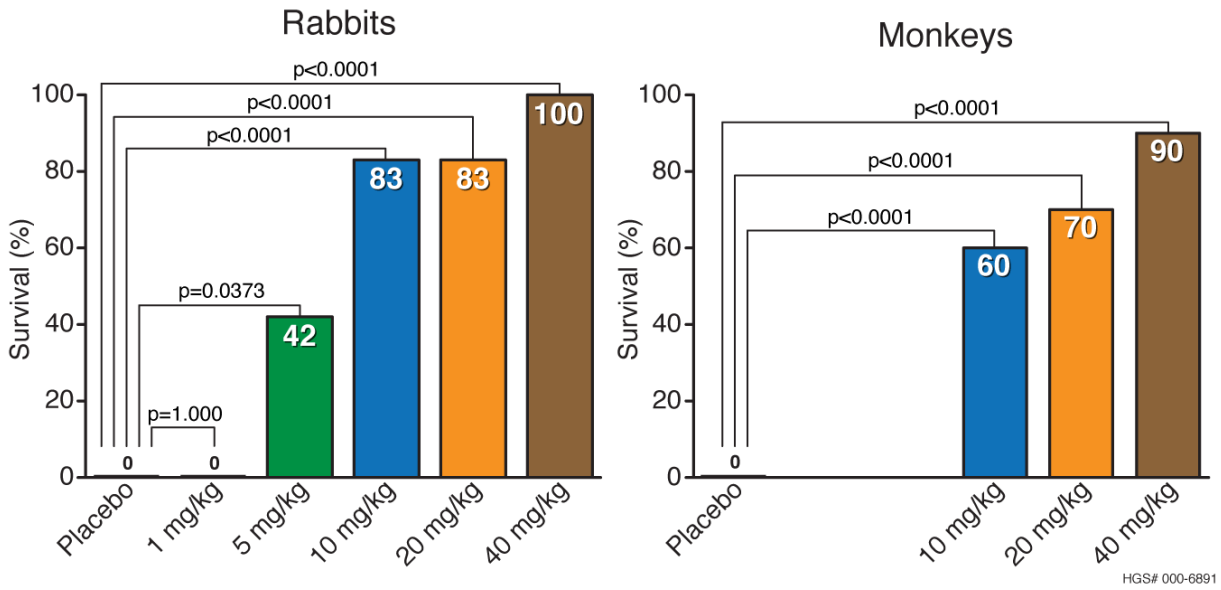

Figure 8-8 **Survival rates in pre-exposure prophylaxis efficacy studies in rabbits (288-HGSIRAB) on left and monkeys (290-N005433) on right**

In the pre-exposure prophylaxis studies, raxibacumab treatment significantly increased survival time compared with placebo and the effect was dose-dependent in rabbits and monkeys (Figure 8-9).

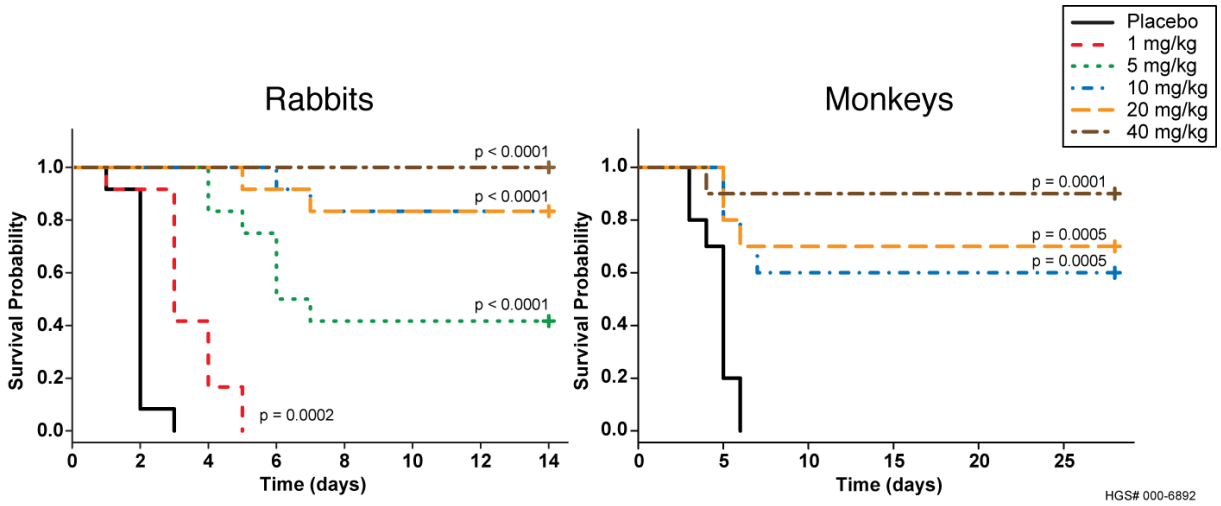

Figure 8-9 **Survival time in pre-exposure prophylaxis efficacy studies in rabbits (288-HGSIRAB) on left and monkeys (290-N005433) on right**

8.3.3 Increased Survival with Earlier Post-Exposure Treatment

Study 358-N005999 was performed to examine the efficacy of raxibacumab when administered post-exposure. This study showed that the raxibacumab survival benefit was greater with earlier treatment indicating that earlier raxibacumab intervention improves outcomes. Raxibacumab provided durable protection in the survivors with no late deaths after the 14-day index period.

As shown in Figure 8-10, the 14-day survival rate was significantly higher in the 2 raxibacumab active groups where the IV injection was administered immediately after spore challenge and 12 hours after challenge (100%), compared with vehicle control group (8.3%), p-value < 0.0001. The 14-day survival rate in the other 2 raxibacumab groups, where the IV injection was administered 24 hours and 36 hours post spore challenge, was 50% and 41.7%, respectively.

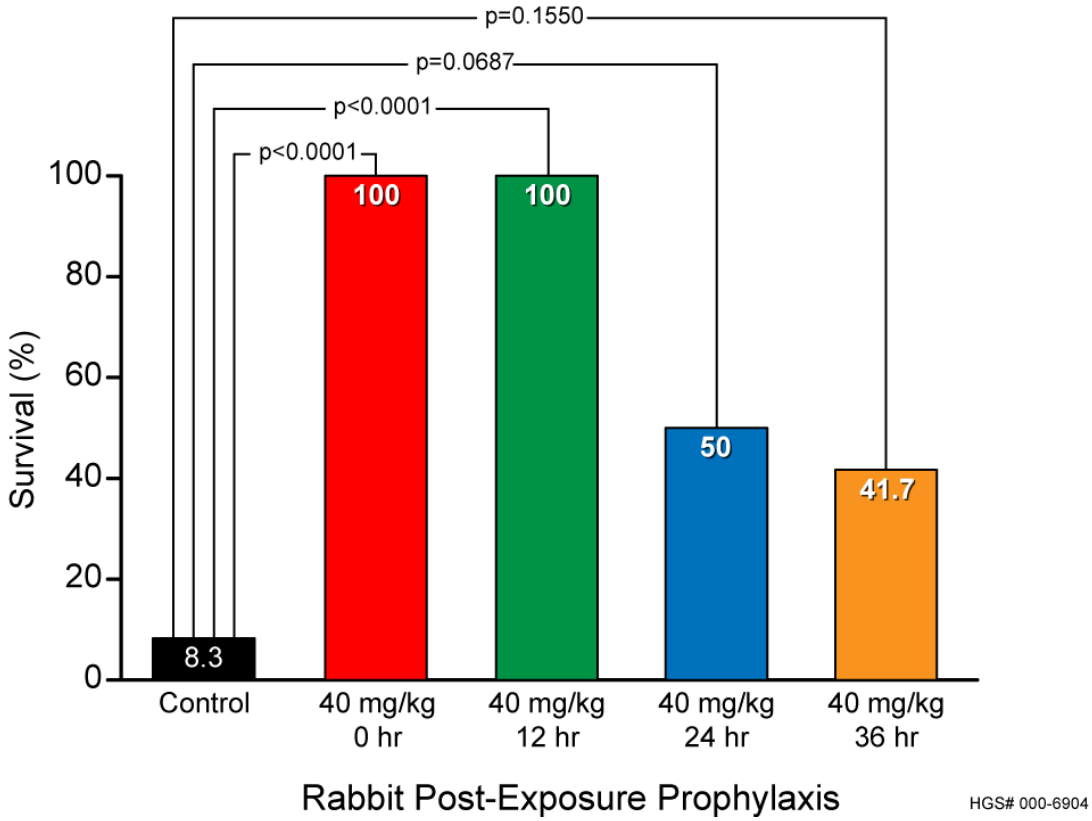

Figure 8-10 **Survival rates when raxibacumab was administered at 0 to 36 hours post spore challenge (358-N005999)**

8.4 Improved Survival Across the Efficacy Studies

The survival rates achieved in the 40 mg/kg raxibacumab dose groups, 100% and 90%, respectively, in Studies 288-HGSIRAB and 290-N005433 were higher than those observed for the same 40 mg/kg dose in the therapeutic treatment studies, 44.4% and 64.3%, respectively, in Studies 682-G005758 and 724-G005829. It is possible that the difference in spore challenge target, 100 x LD_{50} in the prophylaxis studies and 200 x LD_{50} in the therapeutic studies, contributes to this difference. However, the mortality rate in the placebo-treated animals was 100% in all 4 studies confirming that both the 100 and 200 x LD_{50} spore challenges were highly lethal. Moreover at these high LD_{50} levels, there was no statistically significant association between spore challenge dose and the amount of PA expressed at 24 hours based on the data from placebo-treated animals and animals not treated with raxibacumab before 24 hours in Studies 288-HGSIRAB, 615-N104504, 682-G005758, 723-G005835, and 781-G923701.

The more likely explanation for the difference between survival rates between the prophylactic studies and the therapeutic treatment studies is that survival rates decrease as the time from spore challenge to treatment increases. The data from Study 358-N005999 suggests

that this is the case. Of note, the survival rate of 44.4% in Study 682-G005758, where the mean time to treatment was 27 hours, is intermediate to the survival rates of 50% and 41.7% for the groups treated with raxibacumab at 24 and 36 hours post spore challenge, respectively, in Study 358-N005999.

The survival rates in the raxibacumab/antibiotic combination studies (781-G923701 and 789-G923702) were higher than those observed in the raxibacumab monotherapy studies (682-G005758 and 724-G005829). Treatment of animals with human-equivalent doses at the time that the animals have become bacteremic is a setting in which antimicrobials should perform very well. Antimicrobials are active against vegetative bacteria, not spores, so delaying antimicrobial therapy until spores have germinated and animals are bacteremic maximizes the effectiveness of the antibiotics. Prophylactic treatment with antibiotics can lead to late death when spores germinate after antimicrobial therapy has been discontinued (Vietri et al, 2009), while post-exposure treatment with antibiotics eliminates late death. In contrast, anti-toxin therapy would be predicted to be most effective when anti-toxin is present as the toxin emerges. In the time-dependence studies, raxibacumab was 90 to 100% effective when administered at or 12 hours after spore challenge.

8.5 Raxibacumab Does Not Prevent Innate Immune Response to PA

In the rabbit and monkey efficacy studies, animals that survived developed measurable anti-PA antibody concentrations and TNA titers by 14 to 28 days post spore challenge (rabbit levofloxacin studies (723-G005835 and 781-G923701) and monkeys studies (685-G005762, 724-G005829, and 789-G923702). All animals surviving to 28 days had TNA titers higher than 400, which is considered to be protective. Moreover, a rechallenge study in monkeys (374-N006090) showed that the innate immune response was sufficient to protect the raxibacumab-treated surviving animals from a 2nd *B. anthracis* spore challenge approximately 11 months after the 1st challenge (Appendix 13.12).

Humans exposed to *B. anthracis* would also be expected to develop anti-PA antibody and TNA if mortality due to PA toxemia during the 1st month post-exposure can be avoided. For the 6 surviving subjects from the 2001 anthrax attack, anti-PA was 1st detected up to 28 days after likely exposure (Quinn et al, 2004), and in volunteers administered AVA on different schedules, peak anti-PA concentrations occurred 3 to 6 weeks after the 1st vaccine dose (Pittman et al, 2002). Hence, another characteristic of the 40 mg/kg raxibacumab dose in humans is that it maintains protective systemic anti-toxin exposure for a sufficient duration after administration to allow the innate immune response to PA to develop.

9 Translation to Human Dosing

Raxibacumab pharmacokinetics are well understood in rabbits, monkeys and humans and allows translation of effective doses to recommended exposure in humans. Maximum raxibacumab serum concentrations (C_{max}) are critical for survival as the goal is to neutralize PA as rapidly as possible. The C_{max} after a 40 mg/kg IV dose is the same in all 3 species and in anthrax-infected and healthy rabbits and monkeys. Moreover, the steady state volume of distribution (V_{ss}) is similar across the 3 species indicating that systemic exposure is similar in rabbits, monkeys and humans. Biodistribution studies in mice and rats further demonstrate that raxibacumab reaches tissues important in the pathogenesis of anthrax, eg, lungs, lymph nodes and spleen. Humans clear raxibacumab more slowly than monkeys or rabbits, and the longer half-life in humans ensures effective raxibacumab exposures at least as long as in animals. Concomitant administration of raxibacumab with antibiotics did not alter the PK of raxibacumab or the antibiotics, ensuring no decrease in antibiotic exposure or efficacy when the drugs are used in combination. These results suggest that the effective doses in animals are likely to predict effective raxibacumab exposures in humans exposed to anthrax. A single 40 mg/kg dose provides effective, durable protection in animals and, in humans, acheives sufficient exposure to neutralize the maximum PA concentrations observed in animals with inhalation anthrax. Importantly, after a single 40 mg/kg dose, raxibacumab serum concentrations are of a magnitude and duration to provide protection until innate immunity develops

9.1 Raxibacumab PK is Similar across Species

9.1.1 Similar Absorption

Raxibacumab PK in healthy human subjects is compared with raxibacumab PK in healthy and anthrax-infected rabbits and monkeys in Table 9-1 and Table 9-2, respectively. The PK parameter estimates from the human population PK studies are also compared with the noncompartmental PK analysis results for 40 mg/kg IV raxibacumab doses from the human clinical studies, PAM-NH-01, HGS1021-C1064, and HGS1021-C1069 (Table 9-3).

For all 3 species, the initial volume of distribution (V_1) approximates the plasma volume, with the result that maximum plasma or serum drug concentration (C_{max}) for a given dose is similar across the species (918 to 1067 µg/mL at 40 mg/kg). C_{max} does not differ between healthy animals and animals with anthrax. In spore-challenged rabbits and monkeys administered raxibacumab only, clearance (CL) was increased relative to healthy animals, whereas for animals administered raxibacumab with an antibiotic, CL was intermediate to that for healthy animals. These observations suggest that the anthrax disease state affects raxibacumab CL. The lesser impact observed when raxibacumab was administered with antibiotic may be related to the rapid resolution of bacteremia by the antibiotic, which may lead to earlier normalization of organ function, and hence, normalization of raxibacumab CL. CL was faster for rabbits than for monkeys and humans, and human raxibacumab CL was slowest. This implies that for a given dose size, protective raxibacumab concentrations would be maintained for a longer duration in humans than in either rabbits or monkeys. Given the raxibacumab mechanism of action (binding PA to block lethal effects of toxemia) it is reasonable to expect that attaining similar C_{max} should result in similar efficacy in the therapeutic intervention

setting. Hence, a 40 mg/kg dose administered to humans should have efficacy similar to that observed in the animal efficacy studies for that dose.

Examination of individual subject results showed that after a 40 mg/kg raxibacumab IV dose, the lowest C_{max} and $AUC_{0-\infty}$ in human subjects (n = 322) were 589 µg/mL and 8720 µg·day/mL, respectively while the lowest observed C_{max} and $AUC_{0-\infty}$ for surviving spore-challenged rabbits were 405 µg/mL and 1411 µg·day/mL, respectively, and were 385 µg/mL and 2499 µg·day/mL, respectively, for surviving spore-challenged monkeys (Appendix 13.15.1). This indicates that a 40 mg/kg dose to humans can be expected to provide exposure associated with survival for virtually all subjects.

Raxibacumab PK was dose proportional from 1 mg/kg to 40 mg/kg in all 3 species. Given the linearity of raxibacumab PK, the minimum C_{max} and $AUC_{0-\infty}$ for a 20 mg/kg IV raxibacumab dose in a human subject would be about 295 µg/mL and 4360 µg·day/mL, respectively. While the extrapolated minimum $AUC_{0-\infty}$ for a 20 mg/kg dose to humans exceeds the lowest $AUC_{0-\infty}$ associated with survival in spore-challenged rabbits or monkeys, the minimum human C_{max} is lower than the lowest C_{max} associated with survival in rabbits or monkeys. This finding indicates that a proportion of human subjects administered a 20 mg/kg dose would be at risk of not attaining protective serum raxibacumab levels, unlike a 40 mg/kg dose.

9.1.2 Similar Distribution

Formal biodistribution studies have not been performed in humans. However, PK data were collected in all human studies. The steady state volume of distribution (V_{ss}) has been consistent across the studies (58 to 76 mL/kg) and the population PK analysis suggests that raxibacumab distributes in a volume approximately 50% larger than the plasma volume (43 mL/kg, Davies and Morris, 1993) implying distribution to tissues. The V_{ss} in rabbits, monkeys and humans is similar across species and indicates distribution to tissues. Biodistribution studies in rodents with radiolabeled raxibacumab demonstrate that raxibacumab reaches critical target organs for inhalation anthrax disease, including lung, spleen, and lymph nodes (Appendix 13.4).

9.1.3 Metabolism and Elimination

The disappearance of raxibacumab from serum appears to be biphasic with a mean terminal phase elimination half-life of 22 days from the population PK analysis. Mean CL in humans ranged from 2.4 to 2.9 mL/day/kg. These CL values are much smaller than the glomerular filtration rate (2571 mL/day/kg, Davies and Morris, 1993), indicating that, as expected, there is virtually no renal CL of this monoclonal antibody. Because raxibacumab is a fully human monoclonal antibody, it is expected to be metabolized to small peptides and amino acids and should not be affected by or affect small molecule metabolic pathways. Raxibacumab PK has not been evaluated in subjects with renal or hepatic disease.

9.1.4 Effects of Weight, Sex, and Age

In healthy humans and anthrax-infected rabbits and monkeys, body weight was shown to have a significant effect on raxibacumab PK. There was no effect of sex on raxibacumab PK in rabbits, while an effect of sex was not consistently detected in monkeys. There was no effect

of sex, race or age in humans on raxibacumab PK. The size of spore challenge, duration of spore challenge, survival time and survival status were not significant covariates in spore-challenged rabbits or monkeys.

Table 9-1 Raxibacumab PK in rabbits and in healthy humans (mean ± SD)

	Healthy Rabbits		Spore-Challenged Rabbits That Survived			Healthy Humans	
			Study 682-G005758		Study 781-G923701	Population Analysis	
	1 mg/kg (n = 4)	10 mg/kg (n = 4)	20 mg/kg[1] (n = 5)	40 mg/kg[1] (n = 8)	40 mg/kg[2] (n = 18)	40 mg/kg[1] (n = 322)	20 mg/kg[3]
C_{max} (µg/mL)	26 ± 1	276 ± 19	460 ± 51	918 ± 105	929 ± 106	960 ± 164	480 ± 82
$AUC_{0-\infty}$ (µg·day/mL)	174 ± 60	1518 ± 408	1711 ± 323	3504 ± 647	4439 ± 856	16667 ± 3198	8334 ± 1599
$t_{1/2,\alpha}$ (hr)	0.40 ± 0.22	0.24 ± 0.05	0.23 ± 0.04	0.26 ± 0.05	0.09 ± 0.01	1.76 ± 0.36	1.76 ± 0.36
$t_{1/2,\beta}$ (hr)	8.7 ± 4.4	6.9 ± 2.7	3.86 ± 1.11	4.15 ± 1.22	4.58 ± 0.70	22.35 ± 4.04	22.35 ± 4.04
MRT (hr)	12.0 ± 6.0	9.7 ± 3.7	5.41 ± 1.54	5.79 ± 1.69	6.55 ± 0.99	30.09 ± 5.76	30.09 ± 5.76
CL (mL/kg/day)	6.2 ± 1.8	6.9 ± 1.8	11.96 ± 2.36	11.60 ± 2.42	9.35 ± 1.91	2.49 ± 0.49	2.49 ± 0.49
CLD_2 (mL/kg/day)	NA	NA	37.18 ± 1.31	36.73 ± 2.12	86.38 ± 6.74	6.56 ± 0.91	6.56 ± 0.91
V_1 (mL/kg)	39.0 ± 2.1	36.4 ± 2.5	43.56 ± 4.21	43.22 ± 4.53	43.62 ± 5.49	42.86 ± 7.28	42.86 ± 7.28
V_2 (mL/kg)	NA	NA	18.34 ± 4.32	20.70 ± 4.55	16.11 ± 3.16	30.06 ± 4.34	30.06 ± 4.34
V_{ss} (mL/kg)	66.9 ± 9.9	63.2 ± 15.4	61.90 ± 6.45	63.92 ± 7.54	59.73 ± 6.34	72.92 ± 10.07	72.92 ± 10.07

Abbreviations: SD, standard deviation; C_{max}, maximum serum drug concentration; $AUC_{0-\infty}$, area under the serum drug concentration-time curve from time 0 to infinite time; $t_{1/2,\alpha}$, elimination half-life for the 1st phase; $t_{1/2,\beta}$, elimination half-life for the 2nd (terminal) phase; MRT, mean residence time; CL, clearance; CLD_2, intercompartmental clearance; V_1, volume of distribution for the central compartment; V_2, volume of distribution for the peripheral compartment; V_{ss}, volume of distribution at steady-state; NA, not available.

1 Based on individual post hoc estimates.

2 Based on individual post hoc estimates; raxibacumab was administered with levofloxacin.

3 Extrapolated values, assuming linear PK.

Table 9-2 Raxibacumab PK in monkeys and in healthy humans (mean ± SD)

	Healthy Monkeys		Spore-Challenged Monkeys That Survived			Healthy Humans	
			Study 724-G005829		Study 789-G923702	Population Analysis	
	1 mg/kg (n = 4)	10 mg/kg (n = 4)	20 mg/kg[1] (n = 7)	40 mg/kg[1] (n = 9)	40 mg/kg[2] (n = 12)	40 mg/kg[1] (n = 322)	20 mg/kg[3]
C_{max} (µg/mL)	28.8 ± 6.4	261.6 ± 30.3	475 ± 50	1042 ± 177	1067 ± 158	960 ± 164	480 ± 82
$AUC_{0-\infty}$ (µg·day/mL)	267 ± 91	2030 ± 172	3379 ± 655	6544 ± 2400	9903 ± 2279	16667 ± 3198	8334 ± 1599
$t_{1/2,\alpha}$ (h)	1.10 ± 0.84	0.69 ± 0.53	0.69 ± 0.10	0.68 ± 0.14	0.64 ± 0.12	1.76 ± 0.36	1.76 ± 0.36
$t_{1/2,\beta}$ (h)	15.8 ± 4.1	11.8 ± 1.9	10.80 ± 1.79	9.95 ± 2.48	15.27 ± 4.53	22.35 ± 4.04	22.35 ± 4.04
MRT (h)	19.8 ± 4.3	15.8 ± 1.7	14.38 ± 2.64	13.06 ± 3.53	20.78 ± 6.33	30.09 ± 5.76	30.09 ± 5.76
CL (mL/kg/day)	4.1 ± 1.4	5.0 ± 0.4	6.09 ± 1.15	6.64 ± 2.00	4.25 ± 1.05	2.49 ± 0.49	2.49 ± 0.49
CLD_2 (mL/kg/day)	NA	NA	19.95 ± 3.11	19.03 ± 2.97	21.34 ± 2.98	6.56 ± 0.91	6.56 ± 0.91
V_1 (mL/kg)	36.0 ± 7.8	38.6 ± 4.3	42.41 ± 4.81	38.80 ± 6.05	38.18 ± 5.14	42.86 ± 7.28	42.86 ± 7.28
V_2 (mL/kg)	NA	NA	43.04 ± 2.60	42.07 ± 5.83	44.88 ± 7.58	30.06 ± 4.34	30.06 ± 4.34
V_{ss} (mL/kg)	78.8 ± 23.7	78.0 ± 8.3	85.45 ± 6.90	80.87 ± 10.40	83.06 ± 10.08	72.92 ± 10.07	72.92 ± 10.07

Abbreviations: SD, Standard deviation; C_{max}, maximum serum drug concentration; $AUC_{0-\infty}$, area under the serum drug concentration-time curve from time 0 to infinite time; $t_{1/2,\alpha}$, elimination half-life for the 1st phase; $t_{1/2,\beta}$, elimination half-life for the 2nd (terminal) phase; MRT, mean residence time; CL, clearance; CLD_2, intercompartmental clearance; V_1, volume of distribution for the central compartment; V_2, volume of distribution for the peripheral compartment; V_{ss}, volume of distribution at steady-state; NA, not available.

1 Based on individual post hoc estimates.

2 Based on individual post hoc estimates; raxibacumab was administered with ciprofloxacin.

3 Extrapolated values, assuming linear PK.

Table 9-3 Raxibacumab PK parameters by study (Population PK) (mean ± SD)

	Population Analysis[1,2] (n = 322)	PAM-NH-01 (n = 7)	HGS1021-C1064[1] (n = 28)	HGS1021-C1069 (n = 20)
C_{max} (µg/mL)	960 ± 164	1042 ± 88	988 ± 220	979 ± 148
$AUC_{0-\infty}$ (µg·day/mL)	16667 ± 3198	15554 ± 3273	15328 ± 5059	18239 ± 6179
$t_{1/2,\alpha}$ (days)	1.76 ± 0.36	NA	NA	NA
$t_{1/2,\beta}$ (days)	22.35 ± 4.04	16.21 ± 2.30	20.44 ± 6.46	25.68 ± 11.19
MRT (days)	30.09 ± 5.76	22.20 ± 3.65	27.30 ± 8.24	35.09 ± 15.58
CL (mL/day/kg)	2.49 ± 0.49	2.64 ± 0.48	2.85 ± 1.03	2.37 ± 0.63
CLD_2 (mL/day/kg)	6.56 ± 0.91	NA	NA	NA
V_1 (mL/kg)	42.86 ± 7.28	NA	NA	NA
V_2 (mL/kg)	30.06 ± 4.34	NA	NA	NA
V_{ss} (mL/kg)	72.92 ± 10.07	57.6 ± 5.2	71.74 ± 17.36	75.72 ± 11.42

Abbreviations: SD, Standard deviation; C_{max}, maximum serum drug concentration; $AUC_{0-\infty}$, area under the serum drug concentration-time curve from time 0 to infinite time; $t_{1/2,\alpha}$, elimination half-life for the 1st phase; $t_{1/2,\beta}$, elimination half-life for the 2nd (terminal) phase; MRT, mean residence time; CL, clearance; CLD_2, intercompartmental clearance; V_1, volume of distribution for the central compartment; V_2, volume of distribution for the peripheral compartment; V_{ss}, volume of distribution at steady-state; NA, not available.

Mean and SD of individual subjects' post hoc estimates are presented.

1 For group treated with raxibacumab alone.

2 The population PK analyses are based subjects treated with product with the same manufacturing process and formulation proposed for licensure.

9.1.5 PK of Raxibacumab and Antimicrobials Are Unaltered by Coadministration

Because standard therapy for inhalation anthrax includes antimicrobials, the effect of concomitant administration of raxibacumab with ciprofloxacin in humans (HGS1021-C1064) and monkeys (789-G923702) and levofloxacin in rabbits (781-G923701) on the PK of raxibacumab and antimicrobials was studied. Concomitant IV administration of 40 mg/kg raxibacumab with IV and/or PO ciprofloxacin in humans, intragastric ciprofloxacin in monkeys and intragastric levofloxacin in rabbits had little or no effect on the PK of either raxibacumab or the antimicrobials.

9.1.6 PK of Raxibacumab Unaltered by Diphenhydramine

The recommended treatment regimen for raxibacumab includes prophylactic administration of diphenhydramine, therefore, the effect of diphenhydramine on raxibacumab PK was evaluated in the human clinical study HGS1021-C1064. C_{max}/Dose and $AUC_{0-\infty}$/Dose were calculated for the 61 of 86 subjects administered diphenhydramine within 60 minutes prior to raxibacumab administration and the first 25 subjects enrolled in the trial who did not receive prophylactic diphenhydramine. Diphenhydramine use had no impact on C_{max}/Dose or $AUC_{0-\infty}$/Dose indicating that raxibacumab exposure does not appear to differ between subjects who were administered diphenhydramine and those who were not. Diphenhydramine use was

also evaluated as a potential covariate in the raxibacumab population PK analysis, and was found not to impact raxibacumab PK.

9.1.7 Raxibacumab Exposure and PA kinetics

Overall, based on raxibacumab PK in rabbits, monkeys, and humans, as well as serum/plasma PA in *B. anthracis* spore-challenged rabbits and monkeys, it appears that a single 40 mg/kg raxibacumab dose in humans should have efficacy comparable to that observed in the nonclinical therapeutic efficacy studies for the treatment of inhalation anthrax. Evaluation of a 20 mg/kg dose for humans indicates that it would likely be inferior to the 40 mg/kg dose, in that a 20 mg/kg dose would not provide exposures for all subjects greater than or equal to those shown to be associated with survival in the animal efficacy studies, and may not provide sufficient duration of protection for innate immunity to develop. In contrast, a 40 mg/kg dose to humans can be expected to provide all subjects with exposure at least a high as those associated with survival in the nonclinical studies, with a duration of protective raxibacumab concentrations sufficient to allow the development of innate immunity.

Raxibacumab binds the PA of *B. anthracis* and prevents PA-induced toxicity by inhibiting the interaction of PA with its receptor. Because circulating PA is both the target for raxibacumab and the means of anthrax toxicity, the kinetics of PA elaboration and resolution were evaluated in the rabbit and monkey anthrax model characterization studies and the therapeutic efficacy studies. PA levels in untreated or placebo-treated animals were also used to perform population PA kinetic modeling in each of the species.

The population PA kinetic curves for animals that died and that survived within each treatment group are illustrated in Figure 9-1. The serum PA profiles for rabbits and monkeys with anthrax exhibited a triphasic pattern of initial rise, followed by a plateau and then a 2nd rapid rise until death. In contrast animals that were treated with 20 and 40 mg/kg raxibacumab and survived showed the initial rise, but before the plateau phase was attained, serum PA concentrations began to decline. For the surviving animals, the onset of declining serum PA concentrations was generally associated with attainment of negative bacteremia results.

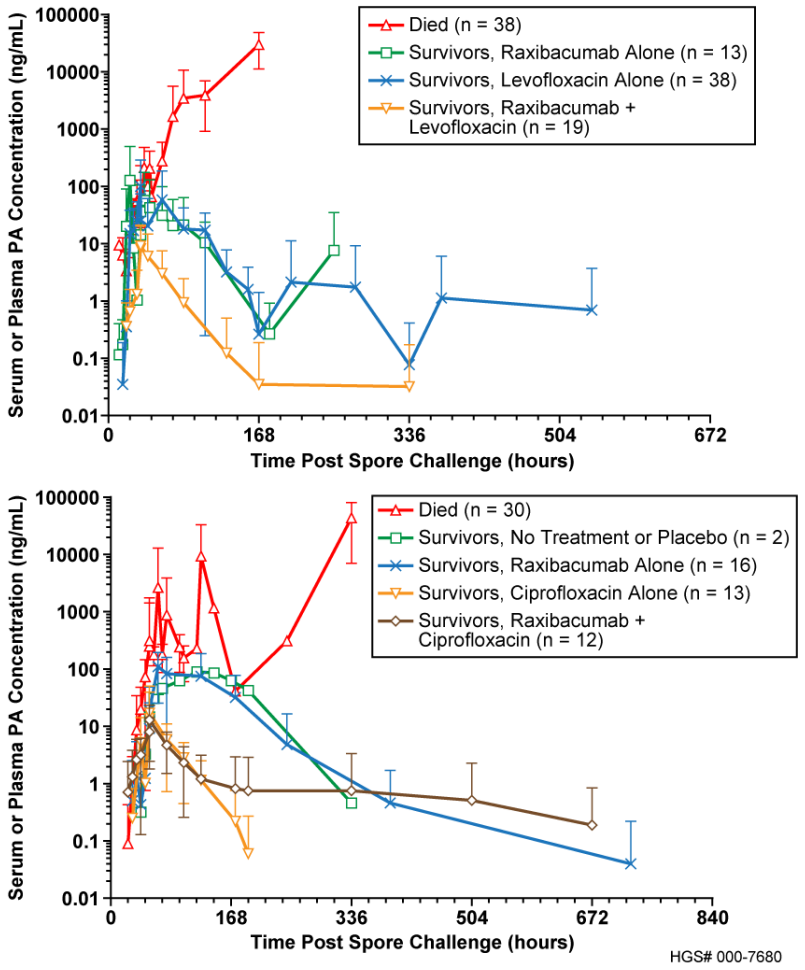

Figure 9-1 Serum PA profiles in rabbits and monkeys

Upper panel, rabbits; lower panel, monkeys

The population PA kinetics analysis was performed using data for 344 specimens from 38 rabbits in 4 studies (615-N104504, 682-G005758, 723-G005835 and 781-G923701) and for 265 specimens from 30 monkeys from 3 studies (685-G005762, 724-G005829 and 789-G923702). All animals used for the PA kinetics analysis were not treated with either raxibacumab or antibiotics, had been exposed to *B. anthracis* inhalation spore challenge and died or were euthanized during the index study period. Body weight, sex, treatment group, and size of spore challenge were among the covariates evaluated for potential effects on PA kinetics, but none of these factors accounted for inter-individual variability in any PA kinetic parameter. Time to 1st bacteremia by culture was associated with λ (the lag time to 1st appearance of serum PA concentrations) consistent with the observation that time to 1st bacteremia coincides with time to 1st detectable serum PA. Survival time was associated with A (the magnitude of serum PA concentrations in the initial plateau phase), λ_2 (the lag time to the 2nd rise) and $\mu_{m,2}$ (the rate of increase of the 2nd rise).

The difference in PA profiles between animals that died and those that survived is relevant for assessing of the efficacy of a human raxibacumab dose. These data show that therapeutic interventions can block the progression of PA concentrations to the high levels associated with death, and that once this is accomplished, PA levels will decrease.

Comparison of the raxibacumab PK results observed for rabbits, monkeys, and humans shows that C_{max} is similar for a 40 mg/kg dose across species. Given the raxibacumab mechanism of action (binding PA to block lethal effects of toxemia), attaining similar C_{max} should result in similar efficacy. Hence, a 40 mg/kg dose administered to humans should have efficacy similar to that observed in the efficacy studies for that dose. After a 40 mg/kg raxibacumab IV dose, all human subjects can be expected to attain exposure associated with survival. For a 20 mg/kg dose, not all subjects would attain exposure associated with survival.

Based on the dissociation equilibrium constant (K_d) determined in vitro, it was determined that a single 40 mg/kg IV raxibacumab dose in humans is adequate to bind at least 99.7% of serum PA for up to 28 days after administration, in at least 95% of subjects.

The highest serum/plasma PA concentrations observed prior to death in control rabbits and monkeys that died were 24,752 and 63,096 ng/mL (298 and 760 nM), respectively. It is assumed that these highest observed serum/plasma PA concentrations in a rabbit and monkey represent the highest levels that might be encountered in a human subject to be treated with raxibacumab. Figure 9-2 illustrates the median and 90% prediction interval serum raxibacumab profiles for a 40 mg/kg single IV dose overlaid with the expected highest PA concentrations to be encountered, both expressed as nM. Following a 40 mg/kg dose, serum raxibacumab levels are equimolar to or greater than the highest expected PA levels for 28 or 48 days, using PA levels from monkeys and rabbits, respectively. In contrast, following a 20 mg/kg dose, serum raxibacumab levels were equimolar to or greater than the highest expected PA levels for 11 to 33 days. Of note, for the 6 surviving subjects from the 2001 anthrax attack, anti-PA immunoglobulin G (IgG) was 1st detected up to 28 days after likely exposure (Quinn et al, 2004), and in volunteers administered AVA on different schedules, peak anti-PA IgG concentrations occurred 3 to 6 weeks after the 1st vaccine dose (Pittman et al, 2002). Since it would be desirable for a human raxibacumab dose to provide protective serum drug levels for at least 28 days to ensure an innate immune response can develop, a 40 mg/kg dose is superior to a 20 mg/kg dose.

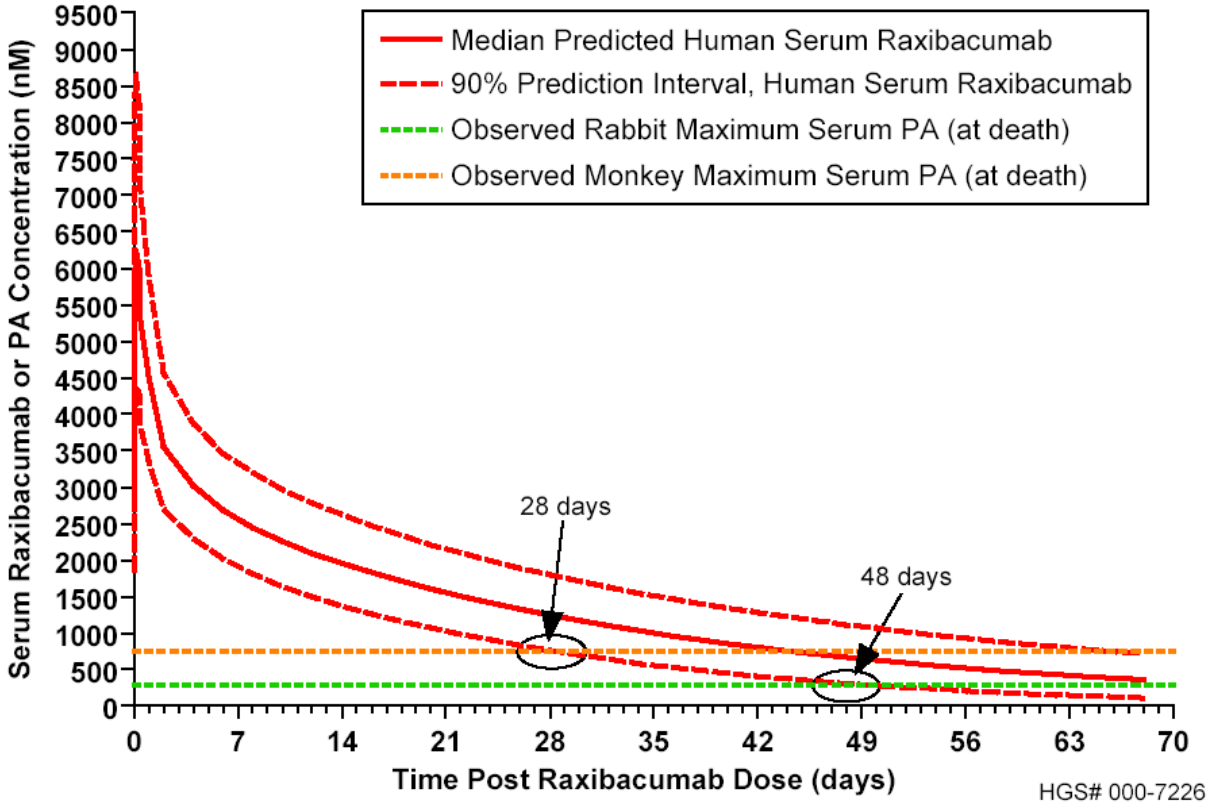

Figure 9-2 **Median and 90% prediction interval serum raxibacumab concentration-time profiles for a single 40 mg/kg raxibacumab IV infusion dose, relative to the highest expected PA concentrations**

10 Safety

Raxibacumab has been administered to over 400 healthy volunteers with a demographic profile representative of the US population. In the 4 human safety studies, raxibacumab was well tolerated with a safety profile similar to that of placebo. A low incidence of mild to moderate rash was observed in Study HGS1021-C1062. These rashes were transient and resolved without medication or with oral diphenhydramine. Prophylactic administration of oral diphenhydramine is recommended with raxibacumab treatment. Raxibacumab has not immunogenic after single administration or after repeat administration either 14 days or greater than 4 months after the 1st dose. Raxibacumab was safe when administered alone or in combination with antibiotics.

In the healthy rabbit and monkey toxicology studies, raxibacumab was safe and well-tolereated with a profile similar to placebo. In anthrax-infected animals, there was a differential histopathology finding in the subgroup of animals that died from anthrax. Compared with placebo-treated animals, in rabbits and monkeys that succumbed to inhalation anthrax despite raxibacumab treatment, histological examination of tissues showed fewer bacteria and less tissue damage in visceral organs and a higher incidence of meningeal findings associated with anthrax infection. This may be a reflection of inability of raxibacumab to cross the blood brain barrier and neutralize the damaging effects of toxins produced by bacteria that reach the brain. No adverse neurological effects were observed in any surviving animal. There were also no adverse effects observed in healthy human subjects receiving raxibacumab nor in surviving animals receiving combination therapy.

10.1 Nonclinical Information Related to Safety

The nonclinical safety assessment program for raxibacumab was designed to support single and multiple dosing in clinical trials in healthy human volunteers. The primary goal of these studies was to evaluate the toxicity associated with multiple dose administration of raxibacumab in healthy animals. Because the target for raxibacumab, PA, is not an endogenous protein, it was predicted that there should be minimal toxicity due to cross-reactivity or exaggerated biologic effect. These studies showed that the safety profile of raxibacumab is similar to that of placebo and are summarized in Section 6.

There was 1 finding of note. In anthrax spore-challenged rabbits and monkeys, the animals that died or were euthanized moribund had findings consistent with anthrax disease by gross necropsy or microscopic examination and the gross and histopathology was qualitatively the same as previously reported for rabbits and monkeys succumbing to inhalation anthrax (Friedlander, 1993; Fritz et al, 1995; Twenhafel et al, 2007; Vasconcelos et al, 2003; Zaucha et al, 1998). The incidence and severity of histopathology findings was the same or greater in the placebo treatment groups for all tissues, except brain, in which the incidence and/or severity of bacteria, hemorrhage and inflammation was greater in the raxibacumab-treated groups. The incidence and severity of brain findings was not greater in the 40 mg/kg raxibacumab treatment group than in the 20 mg/kg raxibacumab treatment group, suggesting that the microscopic findings were not dose-related.

The meningeal findings observed in raxibacumab-treated animals are unlikely to be due to a direct effect of raxibacumab for the following reasons:

1. Biostribuition studies in rodents show that raxibacumab does not reach the brain in healthy animals, although it is found widely in visceral tissues (Appendix 13.4).
2. The tissue-cross reactivity studies performed on rabbit, monkey and human tissues do not show any evidence of raxibacumab binding to brain tissue. (Appendix 13.5)
3. Raxibacumab binds only to soluble PA and does not bind PA once it is bound to cell surface receptors. Consequently, raxibacumab is unlikely to directly cause inflammation or cell death in infected tissues through a mechanism like antibody- dependent cell-mediated cytotoxicity.

Raxibacumab does not cause a general increase in inflammation in the body as evidenced by the observation that the visceral organs of raxibacumab-treated animals tend to have less bacteria, inflammation and hemorrhage than those of placebo-treated animals. Macrophages are not only the primary target of PA-mediated cytotoxicity, they are also the site of spore germination and proliferation. Given the inability of raxibacumab to cross the blood brain barrier, one explanation for the increased brain lesions in raxibacumab-treated animals is that raxibacumab protects macrophages from toxin-induced death in the visceral organs, allowing for more effective infiltration of macrophages in the brain. But because raxibacumab cannot reach the brain, is unable to to prevent the inflammatory effects of toxins produced by bacteria that reach the brain.

It is impossible rule out the potential risk of a drug-disease interaction in animals that succumbed to anthrax; however, the totality of the raxibacumab findings and the published occurrence of meningeal findings in other anthrax-infected animals suggest the finding is more likely a difference in the site of benefit than a deleterious effect. The higher survival rates in the raxibacumab treatment groups in the rabbit and monkey efficacy studies compared with the 100% mortality in the placebo groups suggests that raxibacumab is not exacerbating the clinical outcome of anthrax disease. The observation that the animals that receive raxibacumab and survive have laboratory values (hematology, CRP and temperature) that return to baseline, resolve bacteremia and serum PA, and have no clinical observations at the end of study that indicate residual adverse effects argues against raxibacumab causing central nervous system (CNS) toxicity in animals with anthrax disease. None of the surviving animals had clinical observations of seizures, loss of coordination, or loss of balance at any time or other finsdings suggestive of meningeal involvement during the study or at the end of the observation period. Some of the survivors had remarkable findings (lethargy, not eating, rapid respiration) during the study, but all resolved by the end of the study.

10.2 Safety Evaluation Plan

The safety of raxibacumab has been evaluated in over 400 healthy human volunteers, including over 326 subjects treated at the proposed dose of 40 mg/kg with product manufactured by the process proposed for licensure. These trials evaluated a range of factors established in discussions with FDA as having the potential to impact the safety and immunogenicity profile of raxibacumab, including a range of dose levels (PAM-NH-01),

co-administration with ciprofloxacin (HGS1021-C1064), and repeat dosing prior to (HGS1021-1063) or subsequent to (HGS1021-C1069) clearance of the drug from serum.

Raxibacumab was safe, well tolerated, and no immunogenicity was observed with single or repeat dosing. Concomitant administration of raxibacumab with antibiotics did not alter the safety or PK of either antibiotic or raxibacumab.

10.2.1 Extent of Exposure and Demographics

Per FDA request, to support an indication in therapeutic treatment of inhalation anthrax, a safety database was required comprising at least 300 subjects treated with the raxibacumab product produced by the manufacturing process and in the formulation intended for licensure. The raxibacumab product evaluated in study PAM-NH-01 was manufactured using the M10 manufacturing process while the material evaluated in all subsequent safety studies and animal therapeutic efficacy studies was manufactured using the M11 process which is the process intended for licensure. Consequently, the safety database contains experience with over 300 subjects treated with the recommended dose of raxibacumab 40 mg/kg manufactured by the M11 process and in the formulation proposed for licensure: 86 subjects receiving a single dose from HGS1021-C1064 and 217 subjects receiving a single dose and 23 subjects receiving a double dose from HGS1021-C1064. Twenty of the subjects from the HGS1021-C1064 study received a 2^{nd} dose ≥ 4 months after their initial dose (HGS1021-C1069). These subjects are not re-counted in the total number of subjects receiving raxibacumab (Table 10-1).

Table 10-1 Study agent exposure

	Number of Subjects Exposed (Primary Safety Population shown in bold)								
	Placebo			Raxibacumab				Ciprofloxacin	
Clinical Study	**Single Dose (< 40 mg/kg Raxibacumab Equiv)**	**Single Dose (40 mg/kg Raxibacumab Equiv)**	**Double Dose (40 mg/kg Raxibacumab Equiv)**	**Single Dose < 40 mg/kg**	**Single Dose 40 mg/kg**	**Double Dose 40 mg/kg 14 Days Apart**	**Double Dose 40 mg/kg > 4 Months Apart**	**500 mg PO Twice Daily on Days 1-7**	**2 x 400 mg IV on Day 0 + 500 mg PO Twice Daily on Days 1-7**
PAM-NH-01	23	2	-	73	7	-	-	-	-
HGS1021-C1064	-	-	-	-	**86**[1]	-	-	30	28
HGS1021-C1069	-	-	-	-	-	-	20[6]	-	-
HGS1021-C1063	-	**74**	**6**[4]	-	**217**[2]	**23**[2,3]	-	-	-
Total exposed by dose	23	76	6	73	310	23	20	30	28
Total subjects exposed by study agent		105				406[5]		58	

1 Three subjects received a partial dose.

2 One subject received a partial dose.

3 One subject randomized to the double-dose group received only their 1st dose of raxibacumab and is included with the 217 single-dose subjects.

4 Two subjects randomized to the double-dose group received only their 1st dose of placebo and are included with the 74 single-dose subjects.

5 The 20 subjects from Study HGS1021-C1069 are not included in the total subjects exposed by study agent, as they received their 1st dose of raxibacumab in Study HGS1021-C1064.

6 These 20 subjects from Study HGS1021-C1069 are not counted twice in the primary safety population.

There were a similar number of males and females tested across the studies. Study subjects were predominantly White (66.5%) with representation of Black/African American and Asian race, as well. The median age was 38 years and ranged from 18 to 88 years, with the subjects over 65 years of age contributed by the HGS1021-C1063 study. There were no subjects enrolled under the age of 18 years.

Table 10-2 Demographics and baseline characteristics of subjects in all raxibacumab clinical studies

	Placebo (HGS1021-C1063 + PAM-NH-01) N = 105	Raxibacumab (HGS1021-C1063 + HGS1021-C1064 + HGS1021-C1069 + PAM-NH-01) N = 406	All Studies Combined N = 511
Sex			
Male	64 (61.0 %)	203 (50.0 %)	267 (52.3 %)
Female	41 (39.0 %)	203 (50.0 %)	244 (47.7 %)
Race			
White	76 (72.4 %)	264 (65.0 %)	340 (66.5 %)
Asian	6 (5.7 %)	21 (5.2 %)	27 (5.3 %)
Black or African American	24 (22.9 %)	109 (26.8 %)	133 (26.0 %)
American Indian or Alaska Native	-	2 (0.5 %)	2 (0.4 %)
Native Hawaiian or Other Pacific Islander	-	5 (1.2 %)	5 (1.0 %)
Not Listed	1 (1.0 %)	15 (3.7 %)	16 (3.1 %)
Multiracial	2 (1.9 %)	9 (2.2 %)	11 (2.2 %)
Hispanic or Latino origin	9 (8.6 %)	53 (13.1 %)	62 (12.1 %)
Age (years)			
n	105	406	511
Mean ± SD	40.4 ± 15.4	39.0 ± 14.6	39.3 ± 14.8
Median	40.2	37.3	38.0
Range	(18.1, 78.0)	(18.1, 87.9)	(18.1, 87.9)
Age group			
< 65 years	97 (92.4 %)	384 (94.6 %)	481 (94.1 %)
> = 65 years	8 (7.6 %)	22 (5.4 %)	30 (5.9 %)
Weight (kg)			
n	105	406	511
Mean ± SD	78.2 ± 15.7	77.5 ± 17.3	77.6 ± 17.0
Median	77.8	76.6	76.7
Range	(48.1, 120.1)	(44.6, 163.6)	(44.6, 163.6)

10.3 Treatment-Emergent Adverse Events in the Primary Safety Population

Treatment-emergent AEs are summarized for the primary safety population: those subjects receiving a 40 mg/kg dose of raxibacumab from the manufacturing process and the formulation proposed for licensure, and the placebo-treated subjects in these trials.

The AE profile for raxibacumab is remarkable with an incidence of AEs no greater among the raxibacumab-treated subjects than among placebo-treated subjects. There were no deaths among the raxibacumab-treated subjects and a only a single serious AE (cholecytitis) which the investigator judged most likely due to the subject's pre-existing risk factors.

10.3.1 Treatment-emergent AEs

Treatment-emergent AEs reported in the primary safety population are summarized in Table 10-3. The incidence of AEs was similar in the raxibacumab-treated subjects and the placebo-treated subjects. Morevoer, AEs were no higher among subjects who received a 2nd dose of raxibacumab than those who received a single dose. The incidence of raxibacumab-related AEs was 15%; while investigators reported study agent-related events in 22.5% of the placebo-treated subjects. The incidence of serious and of severe AEs in the raxibacumab-treated subjects was low (0.6% and 1.8%, respectively) and no higher than in the placebo-treated subjects (1.3% and 2.5%, respectively). There was 1 study agent-related serious AE of cholecystitis in a single-dose subject from Study HGS1021-C1063.

Three subjects in the primary safety population experienced a serious adverse event (SAE) (fatal motorcycle accident in 1 double-dose placebo subject, schizophrenia in a single-dose raxibacumab subject with a history of the condition, and cholecystitis in 1 double-dose raxibacumab subject). The SAEs were considered not related to study agent for the first 2 subjects. A relationship between the 1 event of cholecystitis to raxibacumab treatment could not be ruled out, but the investigator judged it to be most likely related to the subject's preexisting rick factors (female gender, obesity, diabetes and hyperlidemia).

Table 10-3 Number of subjects with treatment-emergent AE (primary safety population in bold)

	Placebo Single-Dose N = 74	Placebo Double-Dose N = 6	All Placebo N = 80	Raxibacumab Single-Dose N = 283	Raxibacumab Double-Dose N = 43	All Raxibacumab Treated N = 326
At least 1 AE	32 (43.2%)	6 (100.0%)	**38 (47.5%)**	143 (50.5%)	19 (44.2%)	**162 (49.7%)**
At least 1 related[5] AE	15 (20.3%)	3 (50.0%)	**18 (22.5%)**	46 (16.3%)	3 (7.0%)	**49 (15.0%)**
At least 1 serious AE	-	1 (16.7%)	**1 (1.3%)**	1 (0.4%)	1 (2.3%)	**2 (0.6%)**
At least 1 severe[6]AE	1 (1.4%)	1 (16.7%)	**2 (2.5%)**	5 (1.8%)	1 (2.3%)	**6 (1.8%)**
At least 1 related serious AE	-	-	**-**	-	1 (2.3%)	**1 (0.3%)**
At least 1 related severe AE	-	-	**-**	-	1 (2.3%)	**1 (0.3%)**
At least 1 grade 2 or higher AE	11 (14.9%)	3 (50.0%)	**14 (17.5%)**	37 (13.1%)	8 (18.6%)	**45 (13.8%)**
At least 1 grade 2 or higher related AE	4 (5.4%)	-	**4 (5.0%)**	7 (2.5%)	1 (2.3%)	**8 (2.5%)**

1 Excluded the 20 subjects who received a 2nd raxibacumab dose in HGS1021-C1069. For study HGS1021-C1064, only AEs observed on or after the day of raxibacumab dosing were included. Study HGS1021-C1064 included subjects receiving ciprofloxacin.

2 Included the 20 subjects who received a 2nd raxibacumab dose in HGS1021-C1069.

3 Included all AEs that were observed on or after the date of 2nd raxibacumab dose.

4 Subjects participating in both HGS1021-C1064 and HGS1021-C1069 were counted once only.

5 Possibly, probably or definitely related.

6 Grades 3 and Grade 4 AEs including life-threatening.

10.3.2 AEs in All Subjects in Double-Dose Cohorts

The number of subjects with treatment-emergent AEs in all raxibacumab-treated double-dose cohorts is provided in Table 10-4. Subjects in study HGS1021-C1069 received their 2nd dose of raxibacumab after a ≥ 4 month wash-out period whereas those in study HGS1021-C1063 received their 2 doses 14 days apart. The incidence of AEs among subjects who received 2 raxibacumab doses (44.2%) was similar to that of the overall population of raxibacumab-treated subjects (49.7%, Table 10-3). The incidence of raxibacumab-related AEs in double-dose cohorts was 7.0%. Incidences of at least 1 AE were similar among double-dose subjects in both of these studies. The incidence of at least 1 related AE was 5% in study HGS1021-C1069 compared with 8.7% in study HGS1021-C1063. There was 1 related severe AE (also serious) in Study HGS1021-C1063, as described previously (cholecystitis).

Incidences of AEs and related AEs were similar for subjects who received 2 raxibacumab doses ≥ 4 months apart compared with those who received 2 raxibacumab doses 14 days apart.

Table 10-4 Number of subjects with treatment-emergent AEs in all raxibacumab-treated double-dose cohorts

	HGS1021-C1064 + HGS1021-C1069[1] N = 20	HGS1021-C1063 (Double-Dose Cohorts) N = 23	All Double-Dose Cohorts Combined N = 43
At least 1 AE	9 (45.0%)	10 (43.5%)	19 (44.2%)
At least 1 related[2] AE	1 (5.0%)	2 (8.7%)	3 (7.0%)
At least 1 serious AE	-	1 (4.3%)	1 (2.3%)
At least 1 severe[3]AE	-	1 (4.3%)	1 (2.3%)
At least 1 related serious AE	-	1 (4.3%)	1 (2.3%)
At least 1 related severe AE	-	1 (4.3%)	1 (2.3%)
At least 1 Grade 2 or higher AE	3 (15.0%)	5 (21.7%)	8 (18.6%)
At least 1 Grade 2 or higher related AE	-	1 (4.3%)	1 (2.3%)

1 Subjects participating in both HGS1021-C1064 and HGS1021-C1069 were counted once only.
2 Possibly, probably or definitely related.
3 Grade 3 and Grade 4, including life-threatening.

10.3.3 Common Adverse Events

The number of subjects in the primary safety population with treatment-emergent AEs by Preferred Term ordered by frequency (≥ 1 subject in the placebo or the raxibacumab group) is presented in Table 10-5. The most common AEs across studies were in the system organ class (SOC) category for nervous system disorders contributed by headache, followed by AEs of infections and infestations, and the incident of these types of events was similar in raxibacumab-treated and placebo-treated subjects. These were also the most common AEs among double-dose subjects post-2nd dose. Subjects treated with 2 raxibacumab doses did not have a higher incidence of nervous system disorder AEs as compared with those who received

a single dose, and the shorter interval between raxibacumab doses in study HGS1021-C1063 (14 days as compared to the ≥ 4 month interval between doses in study HGS1021-C1069) did not result in a higher rate of AEs.

Table 10-5 Number of subjects in the primary safety population with treatment-emergent adverse events by MedDRA preferred term ordered by frequency in the raxibacumab group

Preferred Term	All Placebo N = 80	Raxibacumab[1] N = 326
Headache	8 (10.0%)	31 (9.5%)
Upper respiratory tract infection	4 (5.0%)	16 (4.9%)
Nausea	3 (3.8%)	8 (2.5%)
Pain in extremity	1 (1.3%)	7 (2.1%)
Pruritus	-	7 (2.1%)
Diarrhea	2 (2.5%)	6 (1.8%)
Rash	1 (1.3%)	6 (1.8%)
Abdominal pain	2 (2.5%)	5 (1.5%)
Sinusitis	1 (1.3%)	5 (1.5%)
Joint sprain	-	5 (1.5%)
Somnolence	-	5 (1.5%)
Cough	3 (3.8%)	5 (1.5%)
Blood amylase increased	-	4 (1.2%)
Back pain	-	4 (1.2%)
Myalgia	1 (1.3%)	4 (1.2%)
Nasal congestion	-	4 (1.2%)
Urticaria	1 (1.3%)	4 (1.2%)
Vertigo	-	3 (0.9%)
Toothache	-	3 (0.9%)
Fatigue	-	3 (0.9%)
Infusion site pain	-	3 (0.9%)
Edema peripheral	-	3 (0.9%)
Rhinitis	1 (1.3%)	3 (0.9%)
Excoriation	1 (1.3%)	3 (0.9%)
Blood creatine phosphokinase increased	-	3 (0.9%)
Arthralgia	3 (3.8%)	3 (0.9%)
Dizziness	1 (1.3%)	3 (0.9%)
Acne	-	3 (0.9%)
Hypertension	-	3 (0.9%)
Anemia	-	2 (0.6%)
Leukopenia	-	2 (0.6%)
Lymphadenopathy	-	2 (0.6%)
Palpitations	-	2 (0.6%)

Table 10-5 Number of subjects in the primary safety population with treatment-emergent adverse events by MedDRA preferred term ordered by frequency in the raxibacumab group

Preferred Term	All Placebo N = 80	Raxibacumab[1] N = 326
Abdominal pain upper	-	2 (0.6%)
Stomach discomfort	-	2 (0.6%)
Infusion site extravasation	-	2 (0.6%)
Gastroenteritis	1 (1.3%)	2 (0.6%)
Nasopharyngitis	-	2 (0.6%)
Oral herpes	-	2 (0.6%)
Pharyngitis streptococcal	-	2 (0.6%)
Tooth abscess	-	2 (0.6%)
Contusion	1 (1.3%)	2 (0.6%)
Muscle strain	-	2 (0.6%)
Blood pressure increased	-	2 (0.6%)
Prothrombin time prolonged	-	2 (0.6%)
Muscle spasms	-	2 (0.6%)
Syncope vasovagal	-	2 (0.6%)
Insomnia	-	2 (0.6%)
Dysmenorrhea	-	2 (0.6%)
Rhinitis allergic	-	2 (0.6%)
Sinus congestion	-	2 (0.6%)
Dermatitis	1 (1.3%)	2 (0.6%)
Rash erythematous	-	2 (0.6%)
Dyspepsia	2 (2.5%)	1 (0.3%)
Vomiting	2 (2.5%)	1 (0.3%)
Pharyngitis	4 (5.0%)	1 (0.3%)
Leukocytosis	2 (2.5%)	-
Pharyngolaryngeal pain	2 (2.5%)	-
Skin laceration	2 (2.5%)	-
Tension headache	2 (2.5%)	-
Urinary tract infection	2 (2.5%)	-

[1] Subjects participating in both HGS1021-C1064 and HGS1021-C1069 were counted once only. For Study HGS1021-C1064, only AEs observed on or after the day of raxibacumab dosing were included. Study HGS1021-C1064 included subjects receiving ciprofloxacin.

(concluded)

10.3.4 Adverse Events in Subpopulations

The pattern and incidence of AEs was similar between male and female subjects and between White and non-White subjects. Related AEs and AEs of Grade 2 or higher severity were infrequent in all groups. The pattern and incidence of AEs and related was also similar

between subjects < 65 and ≥ 65 years of age. There were no severe, serious, related severe, or related serious AEs among subjects ≥ 65 years of age.

10.3.5 Discontinuations Due to Adverse Events

Across all of the studies there were no subject withdrawals due to AEs. Three subjects in Study HGS1021-C1064 received partial doses of raxibacumab due to mild AEs on the day of raxibacumab dosing: 2 due to mild generalized urticaria, and 1 due to mild left arm and leg clonic muscular contractions. Medication was prescribed only for 1 of the subjects with urticaria; the others resolved without intervention. All events resolved within 1 day and the subjects completed the study.

There were 2 discontinuations of dosing due to AEs in study HGS1021-C1063. One subject in the placebo double-dose group had an AE of moderate skin infection on his left hand on Day 12 (not related, resolved) and did not receive his 2nd placebo dose. This subject completed follow-up. One subject in the raxibacumab single-dose group had his raxibacumab dose discontinued due to an AE of moderate dyspnea (not related, resolved), and received a partial dose. There were no significant changes in vital signs or physical examination, and no evidence of rash, erythema, or edema. No other associated AEs were reported and no significant laboratory abnormalities were found. All symptoms resolved within 20 minutes with no change in physical examination or vital signs during or after this period. Per the investigator, the most likely cause of the subject's symptoms was esophageal spasm caused by rapid eating prior to the infusion and/or anxiousness. This subject completed follow-up.

There were no discontinuations of study agent in Study PAM-NH-01 or HGS1021-C1069.

10.3.6 Long-Term Safety Data

Raxibacumab is intended to be administered as a single 40 mg/kg IV infusion. Follow-up for safety has been for 56 days after administration of raxibacumab (approximately 2.5 half-lives given the raxibacumab half-life [$t_{1/2}$] of 22 days). No longer-term follow up has been performed.

10.4 Adverse Drug Reactions

The most frequent raxibacumab-related AEs in the primary safety population were headache, urticaria, nausea, fatigue, and blood amylase elevation. All raxibacumab-related headaches were mild to moderate in severity, the majority did not occur on the day of dosing, and all resolved, with the majority resolving within 1 day. All raxibacumab-related AEs of urticaria were mild in severity, occurred within 1 day of raxibacumab dosing, and resolved within 1 day. All raxibacumab-related AEs of nausea were mild in severity, occurred within 1 day of dosing, and resolved within 2 days. Three subjects had raxibacumab-related AEs of fatigue, all of which were mild in severity, did not have times of onset that clustered near the day of dosing, and, with the exception of 1 subject whose fatigue was ongoing at the end of the study, all resolved within 2 days. All raxibacumab-related AEs of blood amylase elevation were mild in severity, the majority occurred within 2 days of dosing, and all resolved on-

study, with the exception of 1 subject, who had a raxibacumab related elevated amylase that began 56 days after dosing and was ongoing at the end of the study.

Comparable incidences of related AEs in all SOCs were noted between placebo-treated subjects and raxibacumab-treated subjects in the placebo-controlled studies. Related AEs of ≥ Grade 2 severity were infrequent and the pattern of these AEs was similar among raxibacumab-treated subjects and placebo-treated subjects.

Table 10-6 lists the study-agent related AEs by decreasing frequency. The rates are comparable between the raxibacumab and placebo-treated subjects with no AEs that occurred in more than 1 subject having a higher incidence in raxibacumab-treated subjects than in placebo-treated subjects.

Table 10-6 Number of subjects with study agent (placebo or raxibacumab) related[2] AEs by MedDRA preferred term ordered by frequency in the raxibacumab group

Preferred Term	All Placebo N = 80	Raxibacumab[1] N = 326
Headache	3 (3.8%)	9 (2.8%)
Urticaria	1 (1.3%)	4 (1.2%)
Nausea	2 (2.5%)	3 (0.9%)
Fatigue	-	3 (0.9%)
Blood amylase increased	-	3 (0.9%)
Diarrhoea	1 (1.3%)	2 (0.6%)
Infusion site pain	-	2 (0.6%)
Pain in extremity	-	2 (0.6%)
Dizziness	1 (1.3%)	2 (0.6%)
Rash	-	2 (0.6%)
Rash erythematous	-	2 (0.6%)
Anaemia	-	1 (0.3%)
Leukopenia	-	1 (0.3%)
Neutropenia	1 (1.3%)	1 (0.3%)
Vertigo	-	1 (0.3%)
Stomach discomfort	-	1 (0.3%)
Infusion site phlebitis	-	1 (0.3%)
Malaise	-	1 (0.3%)
Non-cardiac chest pain	-	1 (0.3%)
Edema peripheral	-	1 (0.3%)
Cholecystitis	-	1 (0.3%)
Nasopharyngitis	-	1 (0.3%)
Rhinitis	-	1 (0.3%)
Procedural pain	-	1 (0.3%)

Table 10-6 Number of subjects with study agent (placebo or raxibacumab) related[2] AEs by MedDRA preferred term ordered by frequency in the raxibacumab group

Preferred Term	All Placebo N = 80	Raxibacumab[1] N = 326
Blood pressure increased	-	1 (0.3%)
Arthralgia	1 (1.3%)	1 (0.3%)
Musculoskeletal pain	-	1 (0.3%)
Myalgia	1 (1.3%)	1 (0.3%)
Clonus	-	1 (0.3%)
Lethargy	-	1 (0.3%)
Paraesthesia	-	1 (0.3%)
Somnolence	-	1 (0.3%)
Anxiety	-	1 (0.3%)
Rash macular	-	1 (0.3%)
Rash papular	-	1 (0.3%)
Rash pruritic	-	1 (0.3%)
Skin exfoliation	-	1 (0.3%)
Skin hyperpigmentation	-	1 (0.3%)
Pharmaceutical product complaint	-	1 (0.3%)
Flushing	-	1 (0.3%)
Leukocytosis	1 (1.3%)	-
Tachycardia	1 (1.3%)	-
Abdominal pain	1 (1.3%)	-
Energy increased	1 (1.3%)	-
Pharyngitis	2 (2.5%)	-
Upper respiratory tract infection	1 (1.3%)	-
Muscular weakness	1 (1.3%)	-
Tension headache	1 (1.3%)	-
Cough	1 (1.3%)	-
Hyperhidrosis	1 (1.3%)	-
Pallor	1 (1.3%)	-
Thrombophlebitis superficial	1 (1.3%)	-

[1] Subjects participating in both HGS1021-C1064 and HGS1021-C1069 were only counted once For study HGS1021-C1064, only AEs observed on or after the day of raxibacumab dosing were included. Study HGS1021-C1064 included subjects receiving ciprofloxacin.

[2] Possibly, probably or definitely related.

(concluded)

10.5 Deaths, Other Serious Adverse Events (SAE) and Severe AEs

Across all of the human clinical studies there was 1 death (not related to study agent) in Study HGS1021-C1063 in a subject (placebo double-dose group) who died from injuries sustained in a motor vehicle accident.

Three other subjects experienced SAEs. The SAEs were not related to study agent in 2 of the subjects. The 1 related SAE was a female subject in Study HGS1021-C1063 who received a double raxibacumab dose and experienced cholecystitis (described previously). Two SAEs were reported for 1 subject (moderate pyelonephritis and severe asthma) in Study PAM-NH-01 and were considered not related to study agent. These SAEs occurred 56 days after study agent dosing and resolved within 4 days. Had this subject disclosed her pre-existing conditions of recurrent urinary tract infections and asthma, she would have been ineligible for the study. One subject had a SAE of schizophrenia (Study HGS1021-C1064), which was considered not related to study agent and due to a pre-existing condition.

Severe AEs among placebo-treated subjects included leukocytosis (single-dose placebo) and injury (double-dose placebo). Severe AEs among raxibacumab-treated subjects included cholecystits (double-dose, 14 days apart; also serious; described above), influenza, elevated amylase, prolonged prothombin time (PT), elevated creatine phosphokinase (CK) (related to physical activity not a cardiac event), schizophrenia (also serious, described above) and migraine (all single dose). All severe AEs with the exception of the cholecystitis were not related to study agent.

10.6 Laboratory Evaluations, Vital Signs, and Other Safety Evaluations

10.6.1 Laboratory Evaluations

The rates of ≥ Grade 2 hematology laboratory abnormalities were comparable between placebo-treated subjects and raxibacumab-treated subjects. Grade 3 and Grade 4 laboratory abnormalities were rare, transient and with few exceptions judged unrelated to study agent. There were 2 subjects with Grade 3 leukocytosis (1 placebo and 1 single-dose raxibacumab, both not related to study agent), 1 subject with Grade 4 neutropenia (single-dose raxibacumab; possible related to raxibacumab), and 2 subjects with Grade 3 PT (both single-dose raxibacumab-treated subjects; not related to study agent). All of these laboratory abnormalities resolved on-study. One subject in Study HGS1021-C1069 had fluctuations in PT, which included a shift to Grade 4 PT. PT for this subject returned to baseline levels by the time of an unscheduled post-study assessment. This event was judged to be not related to raxibacumab treatment.

One subject (single-dose raxibacumab) had a transient shift to Grade 3 alanine aminotransferase (ALT) (not related to raxibacumab). There was 1 subject with a transient shift to Grade 3 hyperkalemia (single-dose raxibacumab; not related; not associated with an AE). One subject (single-dose raxibacumab) in Study HGS1021-C1063 had a shift to Grade 3 amylase (not related) and 1 subject (single-dose placebo) in PAM-NH-01 had Grade 3 hyperglycemia.

Shifts from baseline in laboratory parameters were similar among raxibacumab-treated subjects and placebo-treated subjects and no more frequent in double-dose subjects than in single-dose subjects.

10.6.2 Vital Signs

Three subjects treated with a single raxibacumab dose in study HGS1021-C1063 had AEs of mild hypertension, which were judged to be not related to raxibacumab. Two additional subjects in the raxibacumab single-dose group had an AE of mild elevated blood pressure: 1 subject had an AE of mild elevated blood pressure (not related, resolved) and another subject had an AE of mild elevated blood pressure on Day 0 (resolved within 1 day) which was reported as possibly related to study agent. There were no clinically significant abnormalities or AEs of abnormal respirations reported. There were no abnormal findings in temperature other than occasional reports of mild fever, which were not related to study agent and resolved on study. Two AEs of mild tachycardia were reported in study HGS1021-C1063 (resolved). There was no pattern of fever or tachycardia near the time of dosing.

10.6.3 Physical Examination Findings

Any new or worsening findings from the follow-up physical exams were recorded as AEs.

10.6.4 Thyroid Parameters

There were no abnormal findings for thyroid parameters with the single exception of a subject (3 mg/kg IM vastus lateralis) in Study PAM-NH-01 who had a postdose temporal increase in thyroid stimulating hormone levels with normal T_4 levels, consistent with the onset of subclinical hypothyroidism. Subsequent evaluation of the predose blood draw indicated the presence of high titer antibodies to thyroperoxidase, suggesting the presence of a pre-existing thyroid abnormality. The investigator reported the finding as an AE of mild autoimmune thyroiditis not related to study agent.

10.6.5 Rash

The occurrence of rash across all studies is summarized in Table 10-7. Eight (9.1%) subjects in study HGS1021-C1064 experienced mild to moderate infusion-related rashes, none of which were above Grade 2 severity. No other types of infusion reactions were observed and some of the rashes were observed outside the peri-infusion period, but are included in the discussion of infusion-related rashes because they were deemed to be related to raxibacumab administration. There was no consistent pattern suggestive of immune stimulation or an immune complex-mediated process based on the absence of any significant differences in complement levels or cytokine panels. Based on the occurrence of rash in 6 of the first 25 subjects enrolled in the HGS1021-C1064, premedication with oral diphenhydramine was recommended for all subsequently enrolled subjects in the HGS1021-C1064 trial, as well as the HGS1021-C1069 and HGS1021-C1063 trials.

Rash was an infrequent event in study HGS1021-C1063, and the incidence of rash was similar among raxibacumab-treated subjects (2.5%) and placebo-treated subjects (2.5%). Of the 8 incidences of rash, 4 were found to be related to study drug (3 in the raxibacumab

single-dose group and 1 in the placebo double-dose group); all related rashes were transient. Two subjects had a rash that was ongoing at the end of the study (1 subject in the raxibacumab single-dose group and 1 subject in the placebo-double dose group); both ongoing rashes were not related to study drug.

There were no subjects in study HGS1021-C1069 or PAM-NH-01 who reported a rash.

Table 10-7 Occurrence of Rash

Study	Study Agent	Demographics	AE	Onset	Duration	Outcome
HGS1021-C1064						
	PO Ciprofloxacin + raxibacumab[1]	36F/W	Urticaria generalized (mild)	Day 0	1 day	resolved
	Raxibacumab[1]	23F/B	Macular rash trunk (mild)	Day 19	7 days	resolved
	Raxibacumab[1]	38M/B	Rash upper arm (moderate)	Day 0	15 days	resolved
	Raxibacumab[1]	37F/-	Rash chest/abdomen (mild)	Day 5	5 days	resolved
	Raxibacumab[1]	33F/W	Urticaria generalized	Day 0	1 day	resolved
	Raxibacumab[1]	36F/W	Allergic dermatitis [new lotion] (mild)	Day 5	2 days	resolved
	Raxibacumab	38M/W	Rash left arm and leg (mild)	Day 0	1 day	resolved
	IV Ciprofloxacin + raxibacumab	26M/B	Pruritus (mild); rash back and leg (mod)	Day 0; Day 36	2 days; ongoing	resolved; ongoing
HGS1021-C1063						
	Raxibacumab single dose	54F/W	Rash elbow (mild)*	Day 24	1 day	resolved
	Raxibacumab single dose	20F/W	Rash forearm (mild)	Day 9	3 days	resolved
	Raxibacumab single dose	75M/W	Urticaria chest (mild)	Day 0	1 day	resolved
	Raxibacumab single dose	74M/W	Exanthem (mild)	Day 2	2 days	resolved
	Raxibacumab single dose	30F/W	Exanthem (mild)	Day 45	ongoing	ongoing
	Raxibacumab single dose	42M/W	Hand urticaria (mild)	Day 1	1 day	resolved
	Placebo double dose	45F/W	Rash (mild)	Day 21	ongoing	ongoing
	Placebo double dose	46M/W	Urticaria forearm (mild)	Day 14	3 days	resolved

[1] Reported before oral diphenhydramine prophylaxis had been introduced.

10.7 Immunogenicity

There were no subjects who had a positive anti-raxibacumab antibody response in a sensitive assay with a limit of detection of 62.5 ng/mL. Samples were taken for immunogenicity testing predose and postdose at time points up to 28 days after the last dose of raxibacumab.

10.8 Potential Effects on Electrocardiograms and QTc Interval

Electrocardiograms (ECG) were obtained prior to enrollment in all studies, and subjects with clinically significant abnormalities were excluded from participation. Telemetric monitoring was performed in Study HGS1021-C1069 prior to dosing of raxibacumab, during the infusion, and for 2 hours following the infusion. No significant arrhythmias during this period were noted. There were no protocol-required subsequent ECGs obtained after the day of raxibacumab infusion in any study.

10.9 Use in Pregnancy and Lactation

The safety of raxibacumab for use during pregnancy or in nursing mothers has not been demonstrated in clinical trials. A study in pregnant rabbits did not show any detrimental effects to the mother or embryo/fetus. Raxibacumab should be used in pregnancy only if the potential benefit justifies the potential risk to mother and fetus.

Like other human immunoglobulins, raxibacumab is expected to be excreted in milk. The amount of raxibacumab absorbed by the nursing infant is unknown. Because of the potential for adverse reactions in infants nursing from mothers taking raxibacumab, a decision should be made whether to discontinue nursing or to administer the drug, taking into account the importance of the drug to the mother.

10.10 Drug Interactions

Raxibacumab has been administered in combination with ciprofloxacin in monkeys (789-G923702) and humans (HGS1021-C1064) and with levofloxacin in rabbits (781-G923701) and concomitant administration did not affect the efficacy, safety or PK of either raxibacumab or the antimicrobials.

Concomitant use of raxibacumab with diphenhydramine did not alter the safety or PK of raxibacumab (HGS1021-C1064).

10.11 Comparison of Adverse Event Profile with Other Drugs in the Class

Raxibacumab is the 1st product in the class of full human monoclonal antibodies against the PA of *B. anthracis.*

11 Benefit:Risk

Raxibacumab has a favorable benefit:risk profile in the treatment of inhalation anthrax. It fills an important gap in the available treatment options by delivering a potent and specific anti-toxin that acts directly on the primary driver of anthrax pathogenesis. A single 40 mg/kg dose of raxibacumab provides immediate and durable inhibition of anthrax toxin until natural anti-toxin immunity can develop.

Anthrax is a significant bioterrorism threat facing the United States. The attack involving the postal service in 2001 resulted in 11 symptomatic cases of inhalation anthrax. All of these patients suffered serious morbidity requiring prolonged hospitalization and 5 of them died. The US Government has simulated mass anthrax exposures in terror attacks, and the death tolls were of much greater magnitude than sustained in the 2001 attacks.

In response to this danger, the US Government has made the development of a direct anti-toxin a priority for civilian defense. Raxibacumab has met the governmental requirements for inclusion of the SNS and CDC has accepted 20,000 doses and contracted an additional 45,000. With this submission, raxibacumab is the first product developed since the terror attacks to be considered for approval under the Animal Rule. The proposed indication for raxibacumab is for the treatment of patients with anthrax infection. Raxibacumab should be used to treat infections that are proven or strongly suspected to be caused by *B. anthracis* bacteria. When used in combination with antibiotics, raxibacumab is not expected to interfere with antibiotic efficacy. Raxibacumab is recommended for use in combination with antibiotics, but it can be used as monotherapy for patients in whom antibiotics are contraindicated or in whom anthrax disease is due to antibiotic-resistant strains of *B. anthracis*.

The demonstration of efficacy of raxibacumab followed the requirements of the Animal Rule. The design, conduct, and results of the raxibacumab development program met or exceeded each of the 4 requirements of the Animal Rule. In addition, the studies met GLP and statistical requirements typically applied to human trials. Both the 20 mg/kg and 40 mg/kg doses achieved a statistically significant and clinically meaningful difference over placebo, meeting the prespecified primary endpoint of the trials. The statistical analysis plan included prespecified adjustments for multiple comparisons. All of post-hoc sensitivity analyses supported the conclusions of the primary analyses. The 40 mg/kg dose demonstrated statistically significant benefit in every sensitivity analysis. Both the magnitude and the robustness of the results for the 40 mg/kg raxibacumab dose were greater than those for the 20 mg/kg, supporting the selection of the 40 mg/kg raxibacumab dose.

The pivotal studies demonstrated that raxibacumab provides a significant survival benefit over placebo in animals with symptomatic systemic anthrax disease, achieving the primary endpoint. The secondary endpoint of the pivotal studies, demonstration of longer survival time, was also positive. The raxibacumab/antibiotic combination studies also achieved positive results for all of the primary and secondary efficacy endpoints. In addition, the combination studies demonstrated that concomitant treatment with raxibacumab does not block the effect of antibiotics. Additional studies with raxibacumab in pre-exposure

prophylaxis and post-exposure intervention demonstrated that earlier treatment is associated with significantly greater improvement in survival. The efficacy studies also demonstrated that raxibacumab does not prevent the development of an innate immune response and the response in survivors continued to provide protection against anthrax rechallenge 1 year after treatment.

Given the safety and security restrictions limiting access to antibiotic-resistant anthrax, it was not possible to assess the efficacy of raxibacumab against resistant strains. Because its mechanism of action is different from that of antibiotics, raxibacumab is expected to be active against strains resistant to antibiotics. Raxibacumab has been demonstrated to neutralize PA. Based on the site of action of raxibacumab and all available data on the genetic sequences of mutations associated with antibiotic resistance, there is a high likelihood that the activity of raxibacumab would be maintained against all known resistant strains. Further, since the site of action of raxibacumab is preserved in all known strains of the anthrax bacteria and is essential for anthrax pathogenesis, it is unlikely that a naturally-occurring or bioengineered mutation would block the activity of raxibacumab. Therefore, although not demonstrated in the development program, an additional potential benefit of raxibacumab therapy is that it would be effective against antibiotic-resistant strains.

The demonstration of the safety of raxibacumab includes human as well as animal data. The only treatment-emergent adverse event that occurred at a greater rate in healthy human volunteers was a mild to moderate transient rash that was successfully managed with oral antihistamines. In patients who received oral antihistamine prior to raxibacumab infusion, there was no increased incidence of rash compared with placebo treatment. Oral antihistamine prophylaxis is recommended for all patients receiving raxibacumab. Importantly, there was no evidence of immunogenicity associated with raxibacumab administration either as a single dose or with repeat administration.

There were no other safety findings in the human safety studies nor were there any additional safety findings in nonclinical safety studies in healthy animals exposed to single and multiple doses of raxibacumab. There was a potential risk identified only in raxibacumab monotherapy-treated non-surviving animals. Compared with placebo animals, in rabbits and monkeys that succumbed to inhalation anthrax despite raxibacumab treatment, histological examination of tissues shows fewer bacteria and less tissue damage in visceral organs and a higher incidence of meningeal findings associated with anthrax infection. This may be a reflection of the inability of raxibacumab to cross the blood brain barrier and neutralize the damaging effects of toxins produced by bacteria that reach the brain. No adverse neurological effects were observed in any surviving animal and there was no increase in the incidence of adverse effects observed in healthy subjects receiving raxibacumab.

The demonstrated increase in survival rate and survival time constitutes a meaningful clinical benefit in the treatment of a serious life-threatening infection. Raxibacumab is safe and well-tolerated as monotherapy and in combination with antibiotics. The clinically meaningful benefit and favorable safety profile provide a positive benefit:risk profile for the use of raxibacumab in the treatment of anthrax infection.

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13 Appendices

13.1 Appendix 1: In Vitro Pharmacology – Raxibacumab Binding to PA

Raxibacumab binds specifically and with high affinity to PA and inhibits the binding of PA to its receptors. PA binding to its cell surface receptors trigger a cascade of events leading to eventual anthrax toxicity. Inhibiting PA binding to its receptors is a key step in protecting cells from the action of anthrax toxin.

13.1.1 Raxibacumab Binds to PA with High Affinity Binding

PA was immobilized on individual flow cells of a Biacore sensor chip and various concentrations of raxibacumab (4.1 to 333 nM) were injected into the flow cell allowing a 4-minute association phase, followed by a 5-minute dissociation phase. Raxibacumab showed high affinity binding to PA with an equilibrium binding constant (Kd) of 2.78 nM, an on-rate (ka) of 1.99×10^{5} $M^{-1}s^{-1}$, and an off-rate (kd) of 5.54×10^{-4} s^{-1}.

13.1.2 Raxibacumab Potently Inhibits PA Activity

Inhibition of PA binding to soluble anthrax toxin receptor (TEM8) by raxibacumab was measured using a binding inhibition assay. Biotinylated PA was incubated with raxibacumab followed by the addition of flag-tagged receptor; labeled beads were used to develop a signal, which was then measured by electrochemiluminescence (ECL). Raxibacumab potently inhibited PA binding to anthrax toxin receptor TEM8 in a dose-dependent manner with a median inhibitory concentration (IC_{50}) of 503 pM (Figure 13-1).

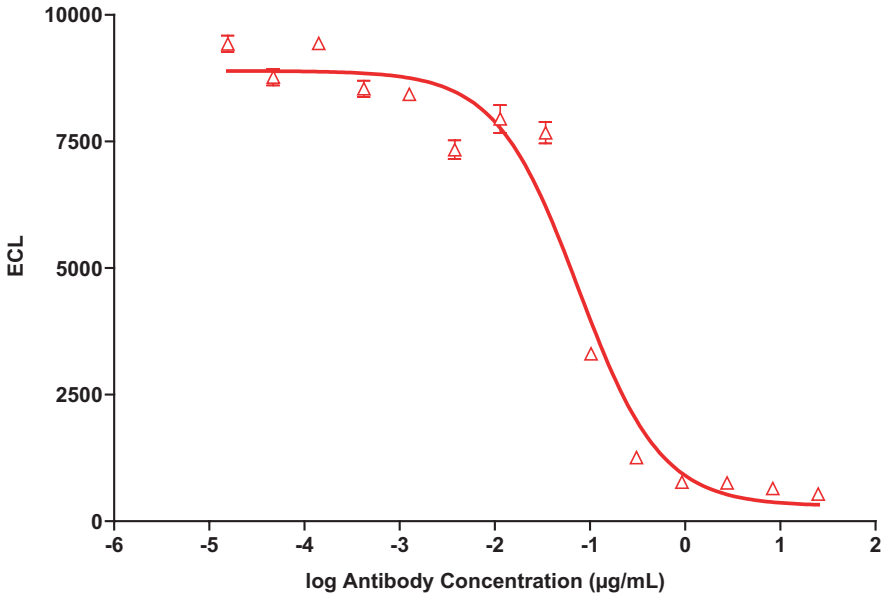

Figure 13-1 Inhibition of PA binding to ATR (TEM8) with raxibacumab

An ECL-based assay was used to measure the inhibition of PA binding to a recombinant soluble anthrax toxin receptor (ATR). PA was pre-incubated with raxibacumab before adding ATR. The starting concentration of raxibacumab was 25 µg/mL and a series of 3-fold dilutions were made to 0.0156 µg/mL. Each sample was tested in triplicate; the mean ± SEM is presented

13.1.3 Raxibacumab Inhibits ^{86}Rb Release in CHO-K1 cells and HumanMacrophages

Following PA binding its receptors, PA molecules assemble into heptamers that form pores on the cell surface through which ^{86}Rb can be released. CHO-K1 cells or human macrophages were pre-loaded with ^{86}Rb and then incubated with PA and raxibacumab. Raxibacumab demonstrated potent inhibitory activity of PA binding to both CHO-K1 cells and human macrophages as demonstrated by preventing ^{86}Rb release. Raxibacumab completely inhibited ^{86}Rb release in both cell types at approximately 5 nM (Figure 13-2 and Figure 13-3).

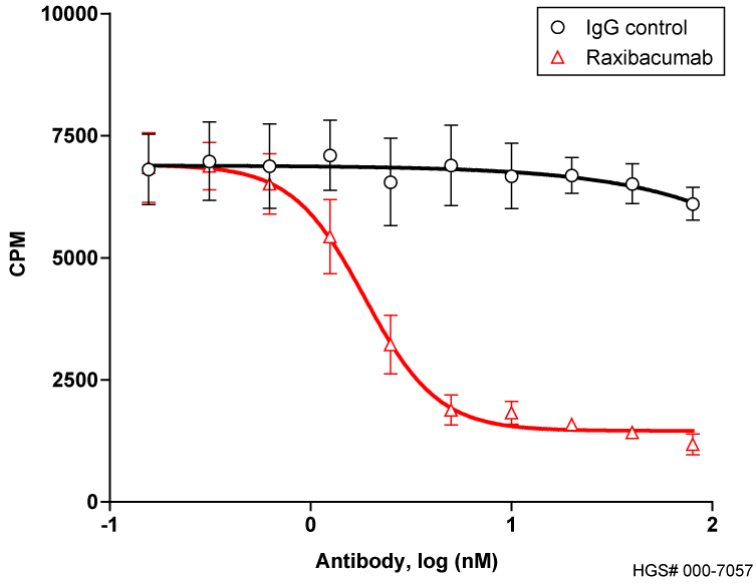

Figure 13-2 Inhibition of ^{86}Rb release from CHO-K1 cells by raxibacumab

Release of ^{86}Rb from CHO-K1 cells was inhibited with raxibacumab, while an IgG_1 control antibody did not show any inhibition.

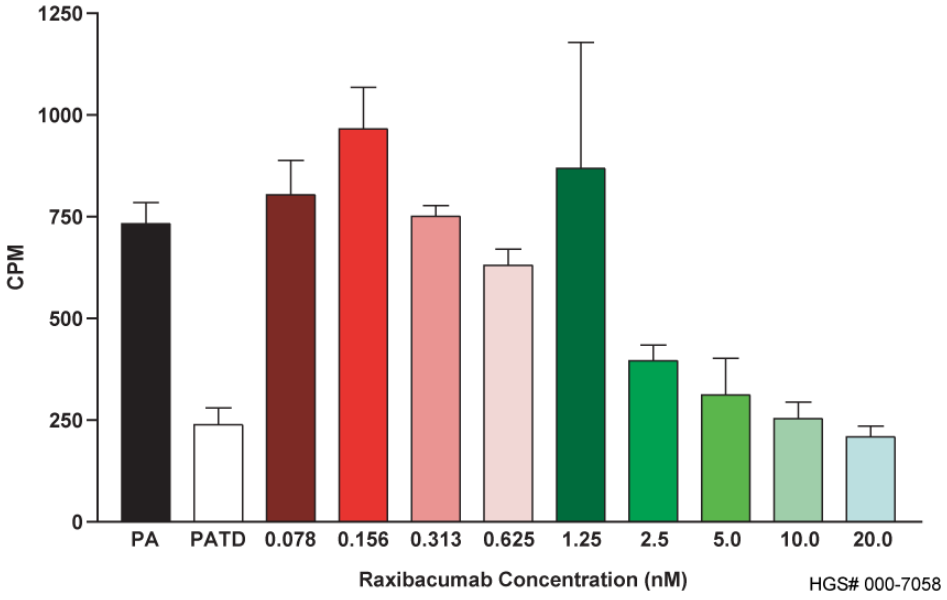

Figure 13-3 Inhibition of ^{86}Rb release from human macrophages with raxibacumab

PA alone served as the positive control; PATD (defective PA) alone was the negative control. The average counts per minute (CPM) in the raxibacumab treatment groups with concentrations of 2.5 nM or above were significantly lower than the average CPM in the PA alone group (all p-value ≤ 0.0063). The inhibition of ^{86}Rb release was dose dependent ($p < 0.0001$).

13.1.4 Raxibacumab Inhibits the Induction of cAMP by PA/EF

A cyclic AMP (cAMP) induction assay using CHO-K1 cells was developed for detecting anthrax-induced intoxication and to evaluate the neutralizing activity of raxibacumab. This assay is based on the principle that EF binds to receptor-bound PA and is shuttled into the cell by receptor-mediated endocytosis. Once EF is internalized, it causes a rise in cAMP that can be detected by enzyme-linked immunosorbent assay (ELISA). Raxibacumab was incubated with PA and EF and then added to cells. After incubation, cells were lysed and measured for cAMP by chemiluminescence. Raxibacumab potently inhibited the induction of cAMP by PA/EF with an IC_{50} of 3.5 nM (Figure 13-4).

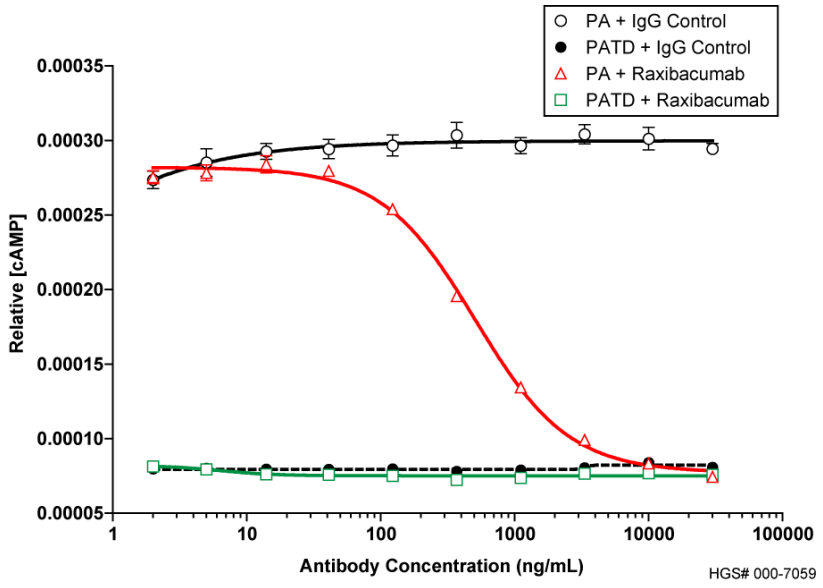

Figure 13-4 Inhibition of cAMP induction by raxibacumab

Raxibacumab or a control IgG_1 antibody were incubated with PA or PATD (defective PA) and EF (edema factor) then added to CHO-K1 cells. Cells were lysed and cAMP induction was detected via chemiluminesence. Using a 4-parameter logistic model, the IC_{50} value was determined to be 509 ng/mL (3.5 nM),

13.1.5 Raxibacumab Inhibits LF-Mediated Cell Killing in Murine Macrophages

A lethal toxin-mediated cell-killing assay with J774A.1 murine macrophages was conducted to investigate raxibacumab inhibition of cytotoxicity caused by lethal toxin (PA/LF). Raxibacumab was pre-incubated. This mixture was then added to macrophages followed by the addition of LF and cell viability was measured. Raxibacumab inhibited lethal toxin-mediated cell death in a dose-dependent manner with an IC_{50} of 0.21 nM (Figure 13-5).

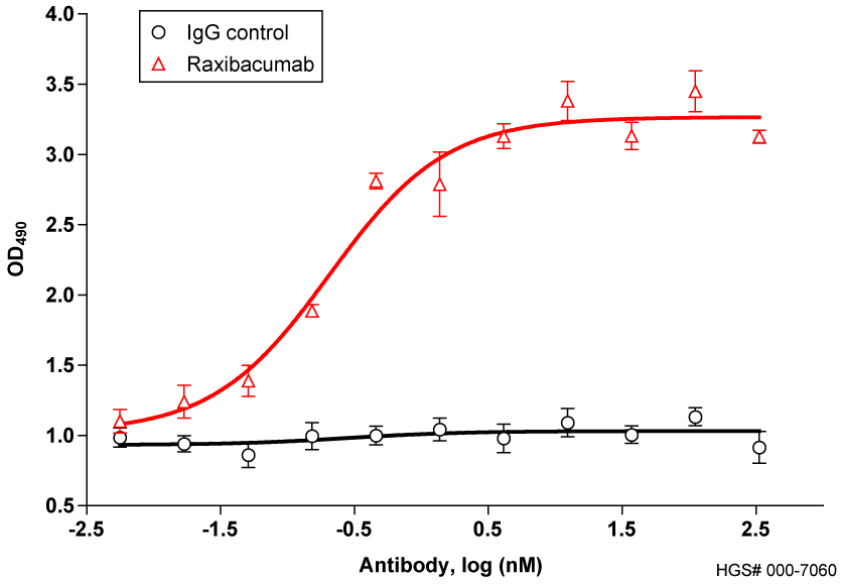

Figure 13-5 Inhibition of lethal toxin-induced cell killing by raxibacumab

Raxibacumab or a control IgG antibody were incubated with PA then added to J774A.1 murine macrophages followed by the addition of LF. Raxibacumab, but not the control antibody, inhibited the macrophage killing.

13.1.6 PA Epitope which Raxibacumab Binds Has Been Identified

Data generated from epitope mapping experiments indicate the region of PA that is recognized by raxibacumab. For matters of security, in this document, HGS is not identifying the precise sequence which is recognized, although the exact epitope has been identified and this epitope is in a highly conserved portion of the PA molecule.

13.1.7 Raxibacumab Binds to PA from Different *B. anthracis* Strains

Supernatants were harvested from cultures of 3 strains of *B. anthracis*, Ames, Sterne, and Vollum, and tested in a quantitative PA ECL-based bridging assay. All of the cultures expressed similar levels of PA (6.8-8.8 µg/mL). Equal volumes of supernatant were immunoprecipitated with raxibacumab or IgG_1 isotype control bound to protein A-agarose beads and the immunoprecipitated proteins were analyzed by western blotting under reducing conditions. As a control, recombinant PA was also immunoprecipitated and loaded on the gel. As shown in Figure 13-6, the PA toxin produced by the Ames, Sterne, and Vollum strains was efficiently bound by raxibacumab.

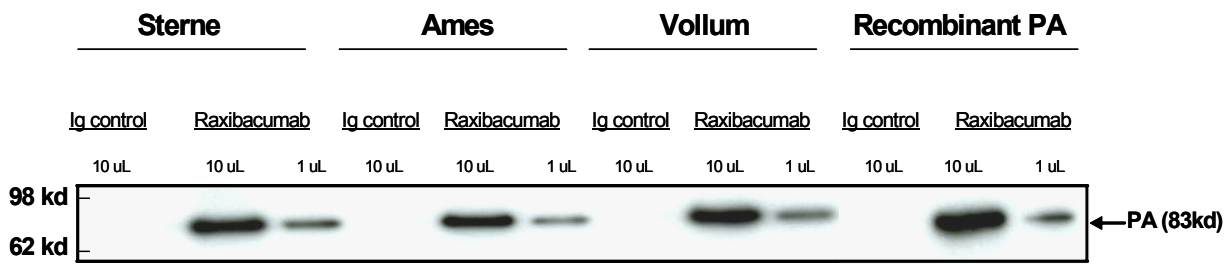

Figure 13-6 Immunoprecipitation of PA-containing supernatants from 3 bacterial strains

Immunoprecipitation was performed using raxibacumab or a non-specific control antibody (IgG_1 control).

These experiments confirm that raxibacumab specifically binds the PA toxin produced by common *B. anthracis* strains of Ames, Sterne, and Vollum. Given the highly conserved sequence of domain IV and its critical role in binding between PA and ATRs, raxibacumab should be able to neutralize the toxicity of PA across a broad range of bacterial strains.

13.2 Appendix 2: In Vivo Pharmacology – Raxibacumab Inhibition of PA Toxicity

Raxibacumab has been shown to provide a survival benefit with pre-exposure prophylaxis, post-exposure prophylaxis, and therapeutic treatment in anthrax lethal toxin models in rats in the studies summarized below.

Raxibacumab also provides a statistically significant and medically important survival benefit in rabbits and monkeys in the pre-exposure prophylaxis, post-exposure intervention, and therapeutic treatment settings. These studies are summarized in Section 8 in the main body of the briefing document, as they are the supportive data for the clinical efficacy claim of treatment of inhalation anthrax in humans.

A summary of each in vivo rodent efficacy study conducted is provided below. For each study, a brief description of the study design is followed by a bulleted list of the key conclusions from the study.

13.2.1 Pre-Exposure Prophylaxis in Lethal Toxin Rat Model

Lethal toxin is the combination of the receptor-binding component, PA, and the metalloprotease, LF, of *B. anthracis* (Friedlander et al, 2001). Based on the in vitro studies, which demonstrated that raxibacumab specifically interferes with the binding of PA to its receptor and prevented killing of cells by lethal toxin, pharmacology studies were extended to an in vivo model where administration of anthrax lethal toxin to rats results in death within 90 minutes. Studies at HGS and reported in the literature have established that Fisher 344 rats are highly susceptible to the lethal effects of systemic doses of lethal toxin (Cui et al, 2004; Ivins et al, 1989; Sellman et al, 2001).

Three studies were performed to evaluate the effect of raxibacumab given by SC, IM, or IV administration at various times before a single injection of lethal toxin in rats. The time to morbundity (TTM) was measured and the number of animals surviving at 24 hours was counted. The survival time, defined as the TTM from the last lethal toxin challenge, was analyzed using the log-rank test. The TTM of the rats that survived the study was censored at 1440 minutes (24 hours). All studies were conducted in male Fisher 344 rats (N = 5 rats/group).

Study 1 treated rats SC, IM, or IV with raxibacumab or an isotype matched control antibody 60 minutes prior to injection of lethal toxin. Animals treated via SC or IM administration received a 10-fold molar excess of raxibacumab to PA and animals treated IV received 0.25 to 10-fold excess raxibacumab.

- Rats treated with raxibacumab SC did not survive to 24 hours and the median TTM was 103 minutes. In the group treated with raxibacumab IM, 4 (80%) rats survived with an average TTM > 1440 minutes (Table 13-1). Both the SC and IM raxibacumab treatment groups showed significantly greater TTM compared with their respective control groups ($p = 0.0027$ and 0.0018, respectively) (Figure 13-7).

Table 13-1 Effect of raxibacumab on TTM of Fisher 344 rats challenged with lethal toxin, SC or IM administration of antibody at t = -60 minutes

Treatment/Route (Excess to PA)	n	Deaths	TTM (minutes)	P-value[1]
IgG control, 1.5 mg/kg SC (10x)	5	5	86	-
IgG control, 1.5 mg/kg IM (10x)	5	5	90	-
Raxibacumab, 1.44 mg/kg SC (10x)	5	5	103	0.0027
Raxibacumab, 1.44 mg/kg IM (10x)	5	1	> 1440	0.0018

[1] Obtained from a log-rank test for the comparison vs raxibacumab groups by the same injection route.

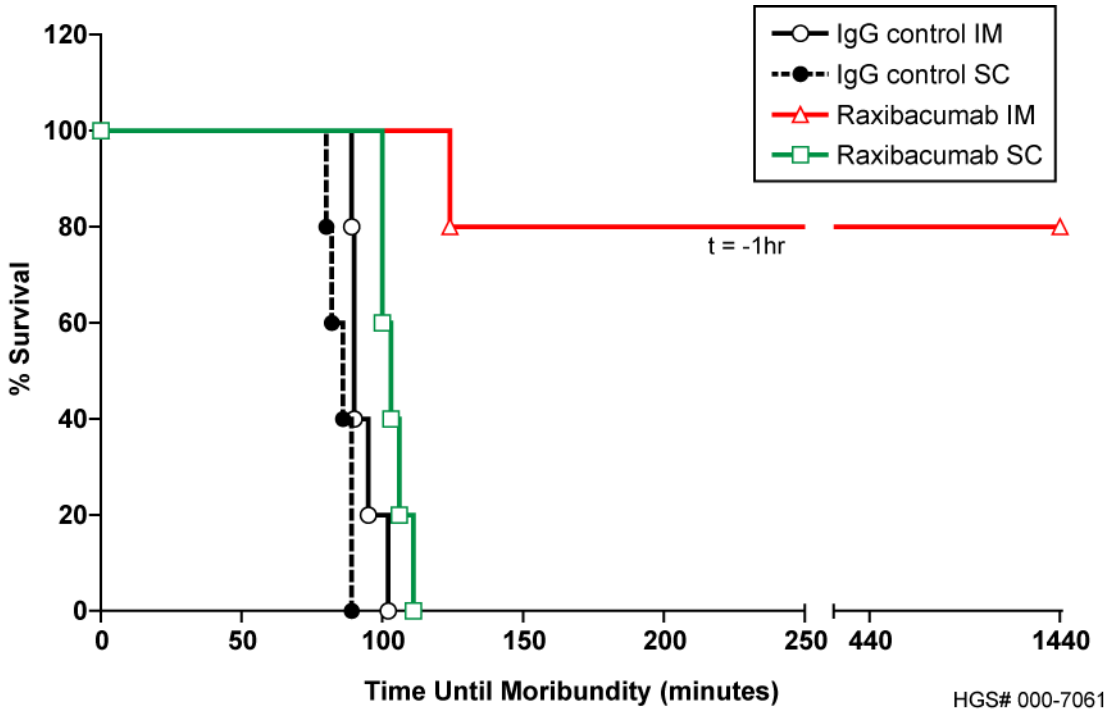

Figure 13-7 Effect of raxibacumab on survival of Fisher 344 rats challenged with lethal toxin, SC or IM administration of antibody at t = -60 minutes

There is a significant difference in the TTM among the rats in these treatment groups (p-value < 0.0001). All rats in the raxibacumab-treated groups had a significantly longer TTM than those in the IgG control-treated groups in the same injection route.

- A single IV injection of raxibacumab (1 to 10-fold molar excess to PA) provided 100% survival compared with the control group (0% survival). Raxibacumab (0.25 or 0.5-fold excess to PA) did not improve overall survival compared with control; however, rats in these groups did show increased TTM (p = 0.0020 for all raxibacumab treatment groups compared with control).

Table 13-2 Effect of raxibacumab on TTM of Fisher 344 rats challenged with lethal toxin, IV administration of antibody at t = -60 minutes

Treatment/Route (Excess to PA)	n	Deaths	TTM (minutes)	P-value[1]
IgG control, 1.5 mg/kg IV (10x)	5	5	63	-
Raxibacumab, 0.039 mg/kg IV (0.25x)	5	5	77	0.0020
Raxibacumab, 0.078 mg/kg IV (0.5x)	5	5	223	0.0020
Raxibacumab, 0.15 mg/kg IV (1x)	5	0	> 1440	0.0020
Raxibacumab, 1.5 mg/kg IV (10x)	5	0	> 1440	0.0020

[1] Obtained from a log-rank test for the comparison vs the IgG control 1.5 mg/kg IV group.

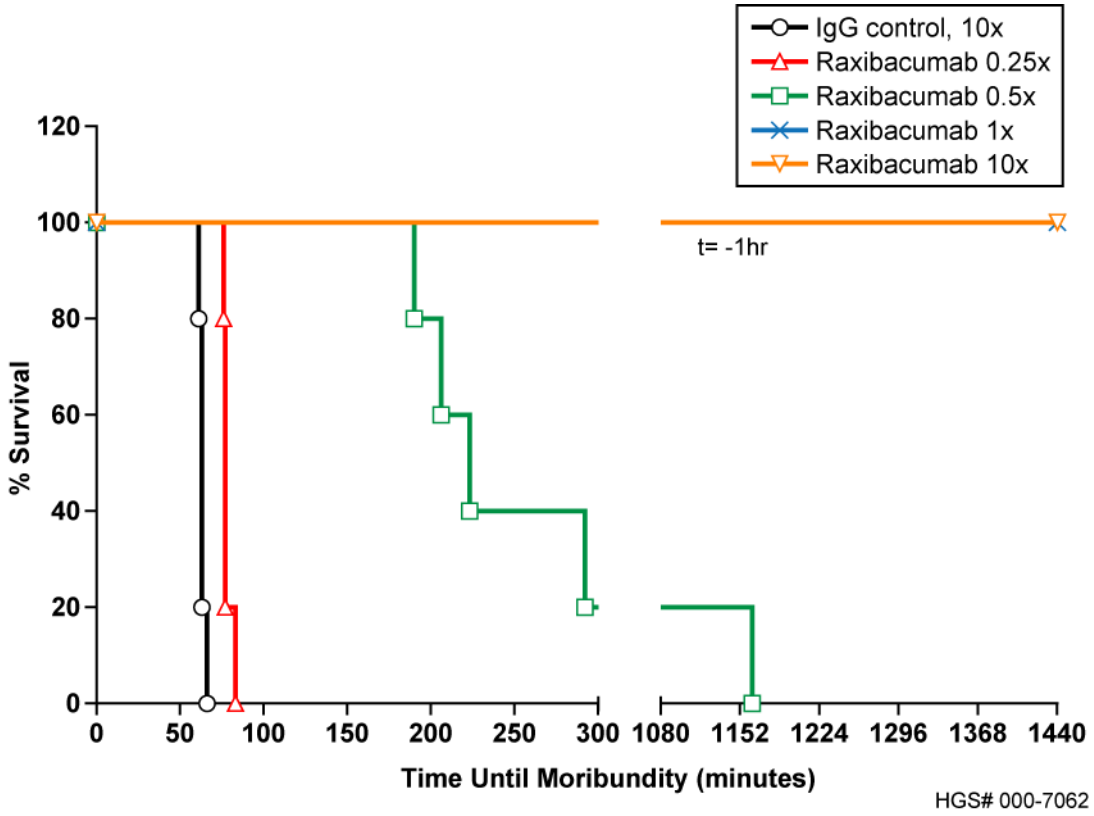

Figure 13-8 Effect of raxibacumab on TTM of Fisher 344 rats challenged with lethal toxin, IV administration of antibody at t = -60 minutes

Antibodies were given IV 60 minutes prior to the lethal toxin challenge. Raxibacumab doses were 0.039-1.5 mg/kg (0.25-10-fold molar excess to PA). There is a significant difference in the survival time among the rats in these treatment groups (p-value < 0.0001). Compared with the rats in the control group, all rats in the raxibacumab-treated groups had significantly longer survival times than those in the control antibody-treated group.

This study demonstrated that a single prophylactic IV administration of raxibacumab given 60 minutes prior to lethal toxin injection at a dose of 1 or 10-fold molar excess over PA provided 100% survival. A single IM administration of raxibacumab provided 80% survival,

while the same dose given SC was not protective. Because raxibacumab may take longer to reach maximum serum concentrations when delivered by the SC or IM route, the experiment was repeated administering raxibacumab 24 hours prior to lethal toxin, as described in the next section.

Study 2 was designed to allow raxibacumab more time to absorb and distribute in the animals and to evaluate the effect on survival. Rats received a single SC, IM, or IV administration of 1.5 mg/kg raxibacumab (10-fold excess to PA) or isotype control antibody 24 hours prior to lethal toxin injection.

- A single SC, IM, or IV injection of raxibacumab 24 hours before lethal toxin injection provided 100% survival, in contrast to control antibody, which afforded no survival at 24 hours.
- All rats treated with raxibacumab had a significantly longer TTM compared with control ($p = 0.0018$). All raxibacumab-treated animals survived the study (24 hours), while the animals in the control group died within a median time of 90 minutes.

Table 13-3 Effect of raxibacumab on TTM of Fisher 344 rats challenged with lethal toxin, SC, IM or IV administration of antibody at t = -24 hours

Treatment/Route	n	Deaths	TTM (minutes)	P-value[1]
IgG control, 1.5 mg/kg SC	5	5	88	-
IgG control, 1.5 mg/kg IM	5	5	90	-
IgG control, 1.5 mg/kg IV	5	5	90	-
Raxibacumab, 1.5 mg/kg SC	5	0	> 1440	0.0018
Raxibacumab, 1.5 mg/kg IM	5	0	> 1440	0.0018
Raxibacumab, 1.5 mg/kg IV	5	0	> 1440	0.0018

[1] From a log-rank test for the comparison vs IgG control 1.5 mg/kg group by the same injection route.

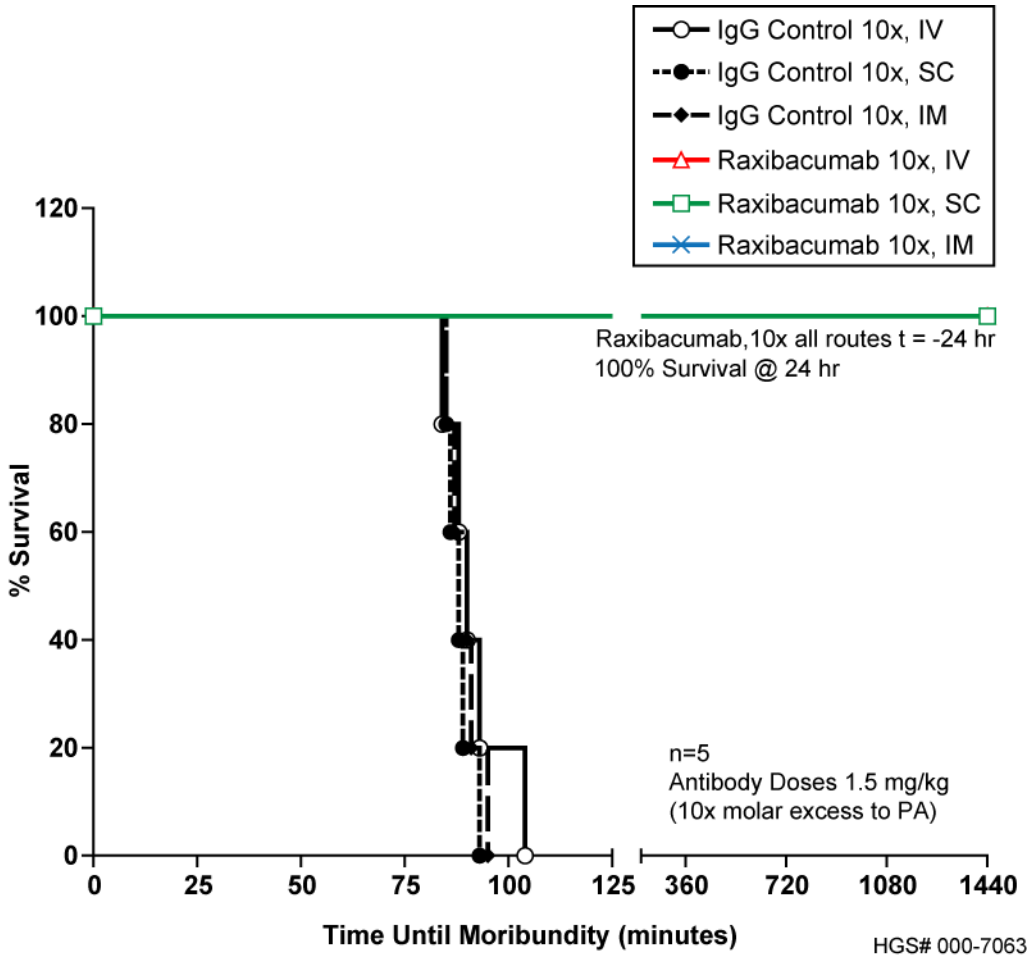

Figure 13-9 Effect of raxibacumab on survival of Fisher 344 rats challenged with lethal toxin; IV, SC or IM administration of antibody at t = -24 hours

Antibodies were given IV, SC, or IM 24 hours prior to the lethal toxin challenge. Antibody doses were 1.5 mg/kg (10x molar excess to PA). There is a significant difference in the survival time among the rats in these treatment groups (p-value < 0.0001). Compared with the rats in the IgG control groups, all rats in the raxibacumab-treated groups had a significantly longer TTM than those in the IgG control treated groups in the same injection route (all p-values = 0.0018).

The data from this study demonstrate that raxibacumab, regardless of route of administration, provides complete protection against mortality when given 24 hours before anthrax lethal toxin challenge. These data prompted an investigation of how long raxibacumab could be administered prior to lethal toxin and continue to provide protection, as described in the next section.

Study 3 investigated how long raxibacumab could be administered prior to lethal toxin challenge while continuing to provide protection against anthrax lethality. Rats received

~1 mg/kg raxibacumab (10-fold molar excess to PA) or isotype control antibody IV 2, 3, 4, 5, 6, 7, or 8 weeks before lethal toxin injection.

- Rats treated with raxibacumab 2 or 3 weeks before lethal toxin challenge showed a 100% survival rate at 24 hours. Rats treated with raxibacumab 4 or 5 weeks prior to challenge showed 80% or 20% survival, respectively (Figure 13-10). All rats treated with control antibody or with raxibacumab 6 or more weeks prior to challenge showed 0% survival.
- A single IV administration of raxibacumab completely protected rats for a minimum of 3 weeks from the lethal toxin-induced mortality and protected 80% of the animals that were challenged 4 weeks after the antibody administration. At Week 5 through Week 8, raxibacumab continued to provide a beneficial effect as evidenced by a longer TTM compared with the control group, although the effect diminished over time. All raxibacumab treatment groups had a statistically significant increase in TTM compared with control ($p \leq 0.0023$).

Table 13-4 Effect of raxibacumab on TTM of Fisher 344 rats challenged with lethal toxin, IV administration of antibody at t = -2 to -8 weeks

Treatment/Time Prior to LT	n	Deaths	TTM (minutes)	P-value[1]
IgG Control, 2 weeks	5	5	77	-
IgG Control, 3 weeks	5	5	86	-
IgG Control, 4 weeks	5	5	84	-
IgG Control, 5 weeks	5	5	92	-
IgG Control, 6 weeks	5	5	93	-
IgG Control, 7 weeks	5	5	91	-
IgG Control, 8 weeks	5	5	85	-
Raxibacumab, 2 weeks	5	0	> 1440	0.0015
Raxibacumab, 3 weeks	5	0	> 1440	0.0018
Raxibacumab, 4 weeks	5	1	> 1440	0.0023
Raxibacumab, 5 weeks	5	4	274	0.0018
Raxibacumab, 6 weeks	5	5	173	0.0021
Raxibacumab, 7 weeks	5	5	140	0.0018
Raxibacumab, 8 weeks	5	5	111	0.0018

[1] From a log-rank test for the comparison vs IgG control group that was administrated at the same time.

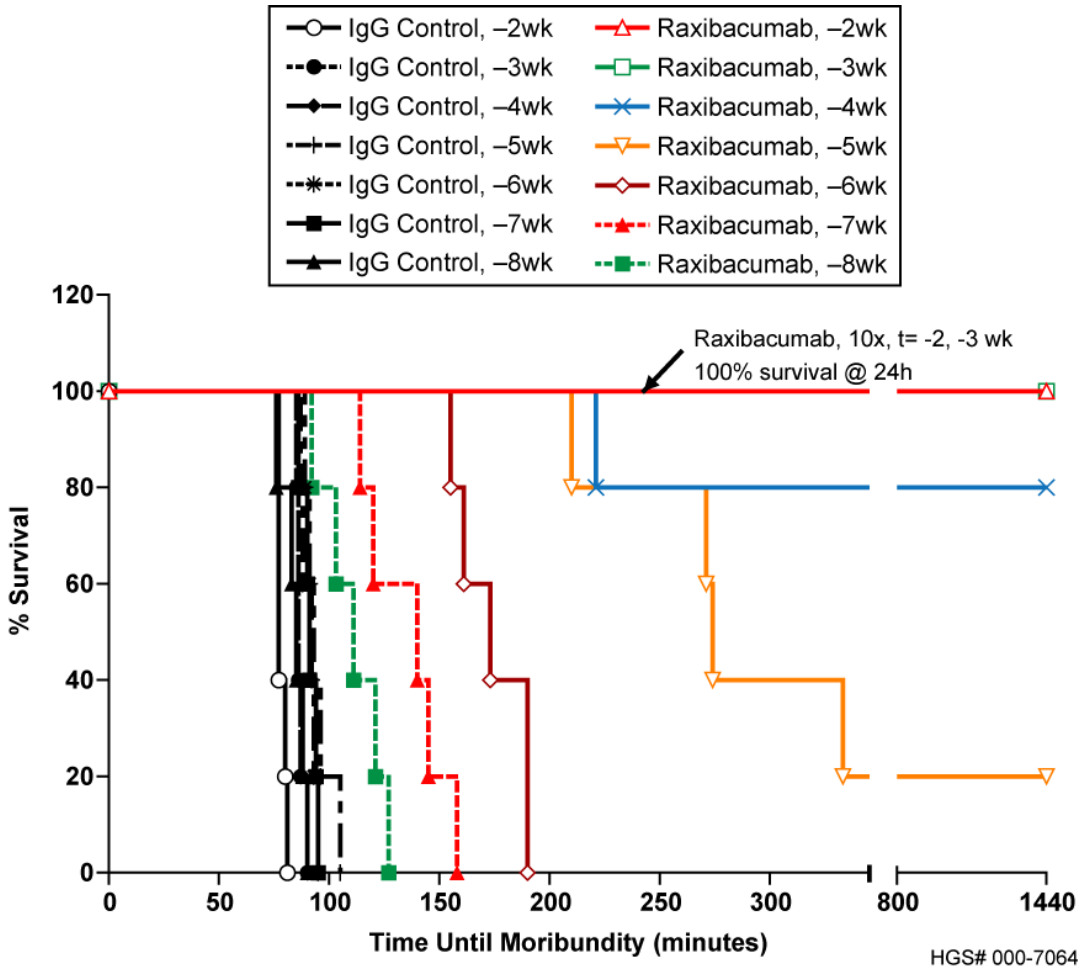

Figure 13-10 Effect of raxibacumab on survival of Fisher 344 rats challenged with lethal toxin, IV administration of antibody at t = -2 to -8 weeks

Antibodies were administered IV at 0.9 mg/kg (10x molar excess to PA) 2 to 8 weeks prior to lethal toxin exposure. The rats in the raxibacumab-treated groups lived significantly longer than those in IgG control groups at all treatment injection schedules (all p-value ≤ 0.0023).

13.2.2 Post-Exposure Treatment in Lethal Toxin Rat Model

A similar rat lethal toxin model was used to examine post-exposure treatment with raxibacumab in rats who have already demonstrated hemodynamic changes in response to a continuous infusion of lethal toxin. The design and results of this study, performed at the National Institutes of Health (NIH), were published (Cui et al, 2005). These investigators concluded that in rats receiving a continuous 24-hour infusion of lethal toxin, raxibacumab administered up to 6 hours after the initial exposure to lethal toxin significantly increased survival ($p \leq 0.01$) compared with control. Given up to 9 and 12 hours afterward, raxibacumab improved hemodynamic function and survival rates. These findings suggest that, clinically, raxibacumab may reduce morbidity and mortality due to anthrax toxin even if administered in subjects who present after the onset of septic shock due to infection with *B. anthracis*

13.3 Appendix 3: PK in Healthy Mice and Rats and Rabbit and Monkey Toxicology Studies

The PK of single-dose raxibacumab was initially characterized in healthy mice and rats Repeat-dose toxicokinetics (TK) of raxibacumab were evaluated in healthy monkeys and rabbits as part of the GLP toxicology studies.

In the 1st single-dose PK study, the PK of raxibacumab was evaluated when administered to normal female BALB/c mice at a dose of 6.75 mg/kg IV, SC, or IM. Clearance (CL) of raxibacumab was 5.1 mL/day/kg following IV administration and was comparable for the IM and SC routes. Following IV injection, the initial (V_1) and steady-state (V_{ss}) volumes of distribution indicated that raxibacumab distributes to the plasma and a fraction of the interstitial fluid space. Maximum serum concentrations (C_{max}) were comparable between the IM and SC routes, but the time to C_{max} (t_{max}) for the IM route (1.1 days) was shorter than the t_{max} for the SC route (2 days). Bioavailability for the IM and SC routes was 107% and 93%, respectively. The terminal elimination half-life ($t_{1/2,z}$) ranged from 6.3 to 8.8 days for the 3 routes of administration.

The 2nd single-dose study was conducted in normal male Fisher 344 rats and 1.5 mg/kg raxibacumab was administered IV or SC. CL of raxibacumab was 8.0 or 8.6 mL/day/kg following IV or SC dosing, respectively. The V_{ss} indicated that raxibacumab distributes to a space larger than the plasma volume and smaller than the extracellular volume in rats. Following SC administration, the t_{max} was 7 days and the bioavailability was 90%. The disappearance of raxibacumab from serum was biphasic, with a $t_{1/2,z}$ of 8.3 or 10.3 days following SC or IV dosing, respectively.

Repeat-dose raxibacumab PK were evaluated in healthy cynomolgus monkeys and NZW rabbits as part of the GLP pivotal repeat dose toxicity study (Study 6962-140) and embryo-fetal toxicity study (Study 6962-173), respectively, to confirm exposure. In the repeat-dose toxicity study in cynomolgus monkeys, raxibacumab was administered SC or IV at a dose of 40 mg/kg on Days 1, 13, and 25. On Day 69, monkeys in the IV dose group also received a single 40 mg/kg raxibacumab dose IM. The overall concentration-time profiles were consistent with the simulated results from a single-dose PK study in healthy monkeys indicating that monkeys were exposed to the intended systemic levels of raxibacumab. Exposure increased with repeat dosing in each group such that C_{max} following the 3rd dose was approximately 1.25 and 1.90-fold higher than C_{max} following the 1st dose in the IV and SC treatment groups, respectively. This is also consistent with the observed $t_{1/2,z}$ of 12.4 to 14 days, when raxibacumab doses are administered once every 12 days. The dose-normalized serum concentration levels indicate that raxibacumab PK appeared to be linear across a 40-fold range (1 to 40 mg/kg) when comparing to results of the single-dose study. No differences across genders were observed. None of the monkeys treated with raxibacumab in this study developed anti-raxibacumab antibodies.

In the embryo-fetal toxicity study, NZW rabbits received 40 or 120 mg/kg raxibacumab IV on gestational days (GD) 7 and 14. Raxibacumab PK in pregnant rabbits was similar to PK observed in healthy non-pregnant rabbits in the single-dose PK study. PK appeared to be

linear over the dose-range evaluated. Exposure increased after the 2nd dose as the prior dose had not yet completely cleared. Treatment-emergent anti-raxibacumab antibodies were detected in 4 rabbits (2 each in the 40 and 120 mg/kg dose groups), which may have affected PK. However, raxibacumab exposure (albeit attenuated) was maintained in these 4 rabbits throughout the study with ≥ 5-fold lower raxibacumab concentrations in the 2 rabbits from the 40 mg/kg group and about 2-fold lower in the 2 rabbits from the 120 mg/kg group.

13.4 Appendix 4: Biostribution in Rodents

A biodistribution study was conducted in healthy rodents. BALB/c mice were injected IV or SC with a single dose of 10 mg/kg ^{111}Indium (^{111}In)-labeled raxibacumab. Biodistribution was determined by both tissue dissection (up to 9 days post-dose) and quantitative whole-body autoradiography in mice. Sprague-Dawley rats were injected IV with a single 1.5 mg/kg ^{111}In-raxibacumab dose primarily to better characterize distribution to lymph nodes. Biodistribution was determined by tissue dissection (up to 1 day post-dose) in rats.

Following SC injection in mice, all the tissues showed a lower concentration of ^{111}In-raxibacumab at 4 hours compared with concentrations achieved following IV dosing due to ongoing absorption. However, by 24 hours after SC dosing, all values reached similar levels as observed following IV administration. The highest concentrations of ^{111}In-raxibacumab were in the plasma and blood, followed by the lungs, kidneys, and liver in both species. The concentrations in these organs declined at a similar rate as was observed for ^{111}In-raxibacumab in the blood. Consistent with the results from the tissue dissection, the lungs, kidney, and liver were the major organs visualized by autoradiography in mice; as expected for a mAb no radioactivity was observed in the brain. Measurable levels of ^{111}In-raxibacumab were found in the lymph nodes as early as 4 hours in mice and 1 hour in rats following dosing, the earliest timepoints evaluated, indicating that raxibacumab enters the lymphatic system and distributes to the lymph nodes. In rats, the concentration of ^{111}In-raxibacumab in the mediastinal lymph nodes was 70-85% of the value in the mesenteric lymph nodes. In mice, the concentrations of ^{111}In-raxibacumab in the liver and spleen increased up to 9 days post dose, suggesting that the liver and spleen are sites of catabolism for ^{111}In-raxibacumab. Precipitation of mouse plasma indicated that > 99% of radioactivity in plasma was protein bound throughout the duration of the study, while the percent of protein-bound radioactivity in urine was much lower, suggesting that raxibacumab is metabolized into smaller peptide fragments that are renally excreted.

13.5 Appendix 5: Tissue Cross-reactivity in Normal Human, Monkey, and Rabbit Tissues

Two ex vivo tissue cross-reactivity studies were conducted with raxibacumab using a full panel of fresh-frozen normal human, cynomolgus monkey, and/or rabbit tissues (see Table 13-5). In the 1st study, raxibacumab was evaluated in cynomolgus monkey and human tissues using product manufactured by a development process (M10) (Study 1494-95). In the 2nd study, product from the M11 manufacturing process proposed for licensure was evaluated in rabbit, cynomolgus monkey, and human tissues (Study IM1634). Raxibacumab or a human IgG_1 control antibody were applied to tissues (3 donors/tissue) at 2.5 or 12.5 µg/mL and immunohistochemically detected using a labeled secondary antibody attached to an unlabeled primary antibody. Bound antibody was visualized with a strepavidin-horseradish peroxidase conjugate and a diaminobenzidine substrate. All slides were evaluated by a pathologist; adequacy of tissue samples was confirmed by CD31 or anti-β_2-microglobulin staining.

Table 13-5 Normal human, monkey, and rabbit tissues evaluated for raxibacumab tissue cross-reactivity

Adrenal	Heart	Salivary gland[3]
Bladder	Kidney (glomerulus, tubule)	Skin
Blood[1]/Blood cells[2]	Liver	Spinal cord
Blood vessels (endothelium)	Lung	Spleen
Bone marrow	Lymph node	Striated muscle
Brain – cerebrum (cortex)	Ovary	Testes
Brain – cerebellum	Pancreas	Thymus
Breast	Parathyroid	Thyroid
Colon	Peripheral nerve[2]	Tonsil[4]
Eye	Pituitary	Ureter
Fallopian tube	Placenta	Uterus (cervix)
Gastrointestinal tract	Prostate	Uterus (endometrium)

1 Study 1494-95; included human and monkey tissues.

2 Study IM1634; blood cells include granulocytes, lymphocytes, monocytes, and platelets; monkey and rabbit blood cells were not evaluated.

3 Study IM1634 only; included human, monkey, and rabbit tissues.

4 Tonsil was not evaluated in rabbits.

Consistent with the target (PA) of raxibacumab not being an endogenous protein, there was little cross-reactivity observed in human, monkey, or rabbit tissues. Variably frequent thyroid binding (in cytoplasmic granules within follicular epithelial cells) was observed in humans and monkeys in both of the tissue cross reactivity studies; no staining was observed in rabbit tissues. Specific staining of monkey breast and prostate tissues was also observed in the 1st study. In addition, staining described as non-specific background-like staining (though not observed with control) was observed in monkey and human skeletal muscle; similar weak staining was observed in 1 of 3 section of human endometrium and prostrate. These findings were not replicated in the 2nd study. Most importantly, none of the ex vivo tissue binding

observations translated into adverse events or altered chemistry or laboratory findings in the monkey toxicology study or in the human clinical studies.

13.6 Appendix 6: Repeat-Dose Toxicology Study in Cynomolgus Monkeys

The GLP pivotal repeat-dose toxicity study (Study 6962-140) was conducted in cynomolgus monkeys based on their phylogenetic proximity to humans and because monkeys may be less likely than other species to develop immunogenicity to a fully human mAb following repeated injection. This study supported initiation of the Phase 1 clinical study (PAM-NH-01) in healthy volunteers at doses up to 40 mg/kg IV. In addition, cynomolgus monkeys were 1 of the 2 species in which pivotal efficacy studies were conducted to evaluate raxibacumab as a treatment for inhalation anthrax.

During Phase 1 of the study, monkeys were randomized to 1 of 3 treatment groups (3 male, 3 female per group) and 40 mg/kg raxibacumab was administered IV or SC once every 12 days for 3 doses (Days 1, 13, and 25). Monkeys in the control group received raxibacumab diluent divided equally by volume between the SC and IV routes of administration (ie, monkeys received 2 injections per treatment day). During Phase 2 of the study, monkeys previously treated in the control group or IV raxibacumab group were treated with a single 0 or 40 mg/kg raxibacumab dose IM at Day 69. Monkeys in the IV/IM treatment groups were sacrificed on Day 77, while monkeys in the SC treatment group were observed through 120 days and returned to the colony.

All monkeys survived to the scheduled sacrifice or for the duration of the study with no raxibacumab-related adverse effects noted in clinical observations, food consumption, or body weight. There were no significant alterations of clinical pathology parameters attributed to treatment with raxibacumab. There were observed decreases in red blood cells (RBC), hemoglobin (HGB), and hematocrit (HCT) values for monkeys treated with raxibacumab IV, although pre-dose values for these monkeys were generally lower prior to treatment (Day -4). These lower values persisted throughout the study and were occasionally significantly different from control values. The relationship to treatment is unknown and may be confounded by the numerous blood collections inherent in the design of the study. The values for these parameters were comparable between all groups at Day 50 and between control and the raxibacumab IV/IM group on Day 77. In addition, C3a levels were evaluated as an indicator of complement activation. Increases in C3a levels were observed variably in individual monkeys, but were not more than 2-fold above each animal's pre-dose level and remained within the normal range throughout the study. Therefore, there appeared to be no biologically-relevant complement activation following raxibacumab administration.

The monkeys sacrificed in the IV/IM treatment group had no gross or histopathologic observations attributed to treatment. Organ weights for thyroid with parathyroid and prostate gland did not reveal any meaningful differences between raxibacumab-treated and control monkeys. There was no evidence of muscle irritation attributed to raxibacumab administration. Incidental findings in other tissues were those commonly observed in cynomolgus monkeys and were not associated with treatment.

Raxibacumab was not immunogenic in monkeys. There was 1 positive antibody response in a control monkey at Days 25, 50, and 69 to the Fab portion of raxibacumab, however, the

samples were negative for neutralizing activity and thus the signal observed was likely non-specific. TK analysis confirmed that the intended systemic exposure was achieved for each of the 3 routes of administration. In summary, IV, SC, or IM administration of 40 mg/kg raxibacumab was considered well-tolerated in this study.

13.7 Appendix 7: Embryo-Fetal Toxicology Study in Rabbits

Rabbits were selected for use in the GLP embryo-fetal toxicity study as rabbits have historically been used as a species for evaluation of potentially teratogenic agents and their use is consistent with recommendations in regulatory guidelines (ICH S5(R2), 1993). Further, the NZW rabbits were 1 of the 2 species in which pivotal efficacy studies were conducted to evaluate raxibacumab as a treatment for inhalation anthrax.

In the embryo-fetal toxicity study (Study 6962-173), raxibacumab was administered IV at 0, 40, and 120 mg/kg to pregnant NZW rabbits on gestational days 7 and 14. There were 20 rabbits treated in each of 3 main study groups and an additional 3 rabbits/groups in matched arms used for TK evaluation. All rabbits survived to the scheduled termination at Day 29 with no remarkable treatment-related clinical signs. A significant decrease in mean body weight gain was noted in the raxibacumab-treated rabbits between Days 21 and 24; however, this was not considered an adverse effect because no effect was observed on overall mean body weight or on food consumption. There were no treatment-related maternal necropsy findings. The finding of pale liver noted in one 120 mg/kg rabbit was considered incidental since pale livers were also noted in 2 control rabbits. The pregnancy rate was similar across the 3 groups (95% to 100%), there were no abortions or early deliveries, and all pregnant rabbits had a litter with viable fetuses. The mean number of corpora lutea, implantation sites, mean percent pre-implantation and post-implantation loss, and mean number of live fetuses were similar across all groups, which indicated that treatment with raxibacumab had no effect on embryo or fetal viability. There were no effects of raxibacumab on maternal uterine or corrected body weights.

There were no raxibacumab-related effects on embryo or fetal viability or growth and none of the fetal external, soft tissue, or skeletal anomalies were considered treatment-related. There were no fetal external variations. One malformation of malrotated hindlimbs was noted in a 40 mg/kg fetus, but was not considered treatment-related due to the absence of a dose-response relationship. The total fetal soft tissue variations were similar across all groups. One malformation of persistent truncus arteriosus was noted in a 40 mg/kg fetus, but was not considered treatment-related due to the absence of a dose-response relationship and an incidence that was within the historical control range. Lastly, the total fetal skeletal variations and malformations were similar across all groups. The fetal incidences of 5^{th} sternebra unossified and 13^{th} rudimentary ribs were significantly increased for the 120 mg/kg dose group, however, these findings were not attributed to raxibacumab since the litter incidence was similar to control, the findings were within the historical control range, and there was an absence of a dose-response relationship.

The immunogenic response was low in this study, with 4 of 46 (8.7%) raxibacumab-treated rabbits developing anti-raxibacumab antibodies (2 in each of the 40 and 120 mg/kg dose groups); however, it must be noted that approximately half of the samples in the 120 mg/kg group and 1 sample in the 40 mg/kg group had raxibacumab levels above the assay tolerance limit, which may have impeded the detection of anti-raxibacumab antibodies. TK analysis was also conducted and confirmed that dose-dependent exposure to raxibacumab was attained despite the presence of anti-raxibacumab antibodies in some animals.

In conclusion, the no-observable-adverse-effect level (NOAEL) in this study was 120 mg/kg for maternal toxicity and the no-observable-effect level (NOEL) for embryo-fetal viability, growth, and fetal development (teratogenicity) was also 120 mg/kg, the highest dose evaluated.

13.8 Appendix 8: Animal Models of Inhalation Anthrax

Because evaluation of new treatment options for inhalational anthrax is not possible in controlled clinical trials in humans for ethical reasons, animal models have been investigated and FDA has offered guidance documents (Animal Models – Essential Elements to Address Efficacy under the Animal Rule, 2009; and Inhalational Anthrax (Post-Exposure)-Developing Antimicrobial Drugs, 2002) on animal models to be used to evaluate treatments for inhalation anthrax. The elements to be included in an animal model to support approval under the Animal Rule are shown in Table 13-6. This section describes how the New Zealand white (NZW) rabbit and cynomolgus monkey models of inhalation anthrax address each of the essential elements and support the choice of these models to demonstrate the effectiveness of raxibacumab as a treatment of inhalation anthrax. References are also made to sections in the application which discuss these elements in greater detail.

Table 13-6 Essential elements for animal models used under the Animal Rule

Characteristics of the etiologic agent:
The challenge agent
Pathogenic determinants
Route of exposure
Quantification of exposure
Host susceptibility and response to the etiologic agent
Natural history of the disease:
Time to onset of disease
Time course of progression of disease
Manifestations (signs and symptoms)
Trigger for intervention
Characterization of the medical intervention:
Product class
Mechanism of action
In vitro activity
Activity in disease of similar pathophysiology
PK in unaffected animals/humans
PK in affected animals/humans
PK interactions with medical products likely to be used concomitantly
Synergy or antagonism of medical products likely to be used in combination
Design considerations for the efficacy studies:
Endpoints
Timing of intervention
Route of administration
Dosing regimen
Available safety information

13.8.1 Characteristics of the Etiologic Agent

13.8.1.1 The Challenge Agent

The challenge agent in all of the raxibacumab efficacy studies is the Ames strain of *B. anthracis* and is the same strain as that used in the United States (US) anthrax attacks in 2001. The amino acid sequence of PA from *B. anthracis* is highly conserved across strains (Price et al, 1999) and raxibacumab has been shown to bind to PA from the Sterne and Vollum strains, as well as Ames.

B. anthracis endospores are resistant to drying, heat, ultraviolet light, gamma radiation, and many disinfectants, and it is this hardiness that has allowed anthrax spores to be developed as biologic weapons by producing "weapons-grade" material.

13.8.1.2 Pathogenic Determinants

The mechanism of anthrax toxicity is well-known. The anthrax toxin is a tripartite toxin that contains enzymatic and binding moieties. The lethal factor (LF) and edema factor (EF) proteins function as the enzymatic moiety of the toxin, while the PA protein functions as the binding moiety. The PA protein 1st binds to ATRs (TEM8 or CMG2) on the cell surface and following proteolytic cleavage, multimerizes into a heptameric barrel structure to which LF and EF bind with high affinity. EF induces edema via a cyclic AMP (cAMP)-dependent pathway, while LF inactivates mitogen activated protein-kinase kinases (MAPKK) resulting in a hyperinflammatory condition contributing to hemodynamic alterations that progress to shock and death of infected subjects.

13.8.1.3 Route of Exposure

The route of infection, by spore inhalation, is the same in the rabbit and monkey models as in humans. The small size of anthrax spores (1-2 µm in diameter) facilitates their easy passage through the upper respiratory tract and deposition into the alveolar spaces.

13.8.1.4 Quantification of Exposure

The delivered dose of anthrax spores was quantified for each animal in the efficacy studies and the results presented as a lethal dose for 50% of the population (LD_{50}) value. The mean spore challenge dose for each treatment group in each of the efficacy studies is summarized in Table 13-7 and Table 13-8 for the therapeutic treatment efficacy studies and the pre-exposure prophylaxis and post-exposure intervention efficacy studies, respectively. The target spore challenge doses were 100 x LD_{50} and 200 x LD_{50} for the prophylaxis/intervention and therapeutic studies, respectively, and were achieved. Both target doses were highly lethal resulting in near 100% mortality across the untreated or placebo-treated animals in all studies (Table 13-9 and Table 13-10).

Table 13-7 Demographics in clinical efficacy studies in the therapeutic treatment setting

Study ID	Study Objective	Species	Age	Weight	# Subjects by Arm	Sex M/F	Prior Treatment/ Exposure	Anthrax Challenge (LD_{50})
615-N104504	Characterization	NZW rabbits	Not used as a criterion	3-5 kg	8	4M/4F	naïve	178 ± 57
685-G005762	Characterization	Cynomolgus monkeys	Not used as a criterion	3.1-7.7 kg	8	7M/1F	monkey pox virus	260 ± 108
682-G005758	Pivotal efficacy; therapeutic treatment	NZW rabbits	205 days All born on same day	Mean 3.1 kg 2.7-3.4 kg	Placebo: 17 20 mg/kg raxi: 18 40 mg/kg raxi: 18	9M/8F 10M/8F 10M/8F	naïve	222 ± 31 230 ± 37 233 ± 54
724-G005829	Pivotal efficacy, therapeutic treatment	Cynomolgus monkeys	Mean 3.7 years 2.7-4.5 years	Mean 3.5 kg 2.3-5.1 kg	Placebo: 12 20 mg/kg raxi: 14 40 mg/kg raxi: 14	6M/6F 7M/7F 7M/7F	naïve	198 ± 44 199 ± 52 157 ± 32
781-G923701	Efficacy, therapeutic treatment in combination with antibiotic	NZW rabbits	Mean 275 days 257-292 days	Mean 3.5 kg 3.0-4.0 kg	Placebo: 12 levo: 20 levo/raxi: 20	6M/6F 10M/10F 10M/10F	naïve	325 ± 110 286 ± 65 283 ± 82
789-G923702	Efficacy, therapeutic treatment in combination with antibiotic	Cynomolgus monkeys	Mean 4.2 years 2.9-5.1 years	Mean 3.4 kg 2.5-6.5 kg	Placebo: 12 cipro: 14 cipro/raxi: 14	6M/6F 7M/7F 7M/7F	naïve	228 ± 58 291 ± 98 302 ± 86
723-G005835	Pilot study for levofloxacin PK and survival rate	NZW rabbits	9.9 months All born on the same day	Mean 3.6 kg 3.2-4.3 kg	Control: 3 10 mg/kg levo: 8 25 mg/kg levo: 8 50 mg/kg levo: 8	3M 4M/4F 4M/4F 4M/4F	naïve	665 ± 178 460 ± 100 509 ± 60 486 ± 104

Table 13-8 Demographics in efficacy studies in the pre-exposure prophylaxis and post-exposure intervention setting

Study ID	Study Objective	Species	Age	Weight	# Subjects by Arm	Sex M/F	Prior Exposure/ Treatment	Anthrax Challenge (LD_{50})
288-HGSIRAB	Pre-exposure prophylaxis	NZW rabbits	13-17 weeks	Mean 2.71 kg 2.5-3.5 kg	Placebo 1 mg/kg raxi 5 mg/kg raxi 10 mg/kg raxi 20 mg/kg raxi 40 mg/kg raxi	6M/6F 6M/6F 6M/6F 6M/6F 6M/6F 6M/6F	naïve	175 ± 95 219 ± 91 197 ± 100 181 ± 64 201 ± 48 243 ± 129
290-N005433	Pre-exposure prophylaxis	Cynomolgus monkeys	21-31 months	Mean 3.4 kg 1.9-3.0 kg	Placebo 10 mg/kg raxi 20 mg/kg raxi 40 mg/kg raxi	5M/5F 5M/5F 5M/5F 5M/5F	naïve	180 ± 72 187 ± 52 177 ± 61 194 ± 79
374-N006090	Anthrax spore rechallenge	Cynomolgus monkeys	Age was not a criterion	2.4-5.1 kg	Naïve Survivors	5M/1F 11M/10F	Anthrax survivors of 290-N005433	154 ± 62 179 ± 76
358-N005999	Time-ranging post-exposure prophylaxis	NZW rabbits	18-20 weeks	2.5-3.1 kg	Placebo 40 mg/kg raxi at 0 h 40 mg/kg raxi at 12 h 40 mg/kg raxi at 24 h 40 mg/kg raxi at 36 h	6M/6F 6M/6F 6M/6F 6M/6F 6M/6F	naïve	121 ± 57 95 ± 49 129 ± 58 65 ± 54 106 ± 64
371-N006101	Dose-ranging, post-exposure prophylaxis	NZW rabbits	17-20 weeks	2.5-3.2 kg	Placebo 5 mg/kg raxi at 24 h 10 mg/kg raxi at 24 h 20 mg/kg raxi at 24 h 40 mg/kg raxi at 24 h 20 mg/kg raxi at 36 h	6M/6F 6M/6F 6M/6F 6M/6F 6M/6F 6M/6F	naïve	161 ± 55 113 ± 85 127 ± 88 100 ± 58 150 ± 84 123 ± 76

Table 13-9 Study design and populations in efficacy studies in the therapeutic treatment setting

Study ID	Study Objective	Species	Anthrax Spore Target (LD_{50})	Trigger for Treatment	Number (%) Bacteremic at/before Treatment	Number (%) Bacteremic on Study	Number (%) Toxemic at/before Treatment	Number (%) Toxemic on Study	Survival Rate in Controls
615-N104504	Characterization	NZW rabbits	200 x LD_{50}	No treatment	NA	8/8 (100%)	NA	NA	0/8 (0%)
685-G055762	Characterization	Cynomolgus monkeys	200 x LD_{50}	No treatment	NA	8/8 (100%)	NA	8/8 (100%)	2/8 (25%)
682-G005758	Pivotal efficacy; therapeutic treatment	NZW rabbits	200 x LD_{50}	Systemic disease (serum PA or increased temperature)	46/53 (86.8%)	50/53 (94.3%)	49/53 (92.5%)	51/53 (96.2%)	0/17 (0%)
724-G005829	Confirmatory efficacy, therapeutic treatment	Cynomolgus monkeys	200 x LD_{50}	Systemic disease (serum PA)	35/40 (87.5%)	40/40 (100%)	38/40 (95.0%)	39/40 (97.5%)	0/12 (0%)
781-G923701	Efficacy, therapeutic treatment in combination with antibiotic	NZW rabbits	200 x LD_{50}	Systemic disease (serum PA or increased temperature)	47/52 (90.4%)	49/52 (94.2%)	49/52 (94.2%)	50/52 (96.2%)	0/12 (0%)
789-G923702	Efficacy, therapeutic treatment in combination with antibiotic	NZW rabbits	200 x LD_{50}	Systemic disease (serum PA)	36/40 (90.0%)	38/40 (95.0%)	38/40 (95.0%)	40/40 (100.0%)	0/12 (0%)
723-G005835	Pilot study for levofloxacin PK and survival rate	NZW rabbits	200 x LD_{50}	Systemic disease (increased temperature)	22/24 (91.7%)	25/27 (92.6%)	22/24 (91.7%)	26/27 (96.3%)	0/3 (0%)

Table 13-10 Study design and populations in efficacy studies in the pre-exposure prophylaxis and post-exposure intervention setting

Study ID	Study Objective	Species	Anthrax Spore Target (LD_{50})	Trigger for Treatment	Number (%) Bacteremic at the Time of Death	Survival Rate in Controls
288-HGSIRAB	Pre-exposure prophylaxis	NZW rabbits	100 x LD_{50}	Time relative to spore challenge	28/35 (80.0%)	0/12 (0%)
290-N005433	Pre-exposure prophylaxis	Cynomolgus monkeys	100 x LD_{50}	Time relative to spore challenge	15/18 (83.3%)	0/10 (0%)
374-N006090	Anthrax spore rechallenge	Cynomolgus monkeys	100 x LD_{50}	Time relative to spore challenge	6/6 (100%) (all control animals)	0/6 (0%)
358-N005999	Time-ranging post-exposure prophylaxis	NZW rabbits	100 x LD_{50}	Time relative to spore challenge	17/24 (70.8%)	1/12 (8.3%)
371-N006101	Dose-ranging, post-exposure prophylaxis	NZW rabbits	100 x LD_{50}	Time relative to spore challenge	NA	0/10 (0%)

13.8.2 Host Susceptibility and Response to Etiologic Agent

B. anthracis is a zoonotic infection that is capable of being transmitted from animals to humans. Animal anthrax infection has been documented in a wide range of mammalian species with a high mortality rate. Human infection results from contact with contaminated animals or animal products or from intentional exposure as in the anthrax attacks of 2001. The pathology of the inhalational form of the disease in rabbits and non-human primates is remarkably consistent with that observed in human (Fritz et al, 1995, Zaucha et al, 1998, Phipps et al, 2004; Twenhafel et al, 2007; Vasconcelos et al, 2003). Although subtle differences among species are present, the symptoms, pathological changes, and clinical outcomes in rabbits and non-human primates are very similar to those observed in human disease. The immune response to anthrax infection has been well characterized in both non-human primates and rabbits (Phipps et al, 2004; Zaucha et al, 1998). Consequently, these animal species have been used extensively to test different anthrax antigens and immunization regimens to demonstrate efficacy in protection against lethal infection, and provide the basis for approval of anthrax vaccines for use in humans (Fellows et al, 2001). The non-human primate model also has been valuable for demonstration of the efficacy of antibiotics (quinolones and doxycycline) in post-exposure models of inhalational anthrax (Friedlander et al, 1993; Kao et al, 2006). The following sections enumerate key characteristics of inhalational anthrax infection in the non-human primate and rabbit and highlight the clinical course that leads to high mortality and the toxin-mediated pathology associated with inhalational anthrax infection.

13.8.3 Natural History of Disease

The natural history of inhalation anthrax is the same in rabbits, non-human primates, and humans. Inhaled spores are quickly engulfed by alveolar macrophages and germinate while being transported to the mediastinal and peribronchial lymph nodes. Anthrax bacilli multiply in the lymph nodes, causing hemorrhagic mediastinitis and disseminate throughout the body in the bloodstream. With rapid bacterial replication, toxins enumerated are responsible for much of the pathologic sequelae. Disease progression and manifestations of the disease are similar across rabbits, monkeys, and humans and are described in detail in subsequent sections.

13.8.3.1 Time to Onset of Disease

The development of clinical signs of anthrax in rabbits after spore challenge is reported to be within 24 hours of death, which occurs at a mean time of 2.4 days, making time of disease onset slightly later than 24 hours (Zaucha et al, 1998). For non-human primates, 1st bacteremia presents 2 days before death (Vasconcelos et al, 2003). Because death occurs with a median time of 4 days in non-human primates, onset of disease by 1st detectable bacteremia is approximately 2 days. In the HGS efficacy studies, the median time to onset of disease as evidenced by detectable serum PA or bacteremia (Table 13-11) is consistent with the values reported in the literature for rabbits and non-human primates. Of note, the earlier detection of PA in the rabbit efficacy studies, compared with the rabbit characterization study, may be due to the fact that blood samples were first taken in the rabbit efficacy studies at 12 hours post spore challenge versus 24 hours post spore challenge in the characterization study. The

median incubation period from exposure to onset of symptoms in humans following the anthrax attacks in 2001 was 4 days (range 4-6 days) (Jernigan et al, 2001) similar to that in non-human primates.

Table 13-11 Time to onset of toxemia and bacteremia

Study ID	Study Objective	Species	Anthrax Spore Challenge Target (LD_{50})	Median Time to Toxemia	Mean Time to Bacteremia
615-N104504	Characterization	NZW rabbits	200 x LD_{50}	30 hours	26 hours
682-G005758	Pivotal efficacy, therapeutic treatment	NZW rabbits	200 x LD_{50}	20 hours	24 hours
781-G923701	Efficacy, therapeutic treatment in combination with antibiotic	NZW rabbits	200 x LD_{50}	24 hours	25 hours
685-G005762	Characterization	Cynomolgus monkeys	200 x LD_{50}	39 hours	36 hours
724-G005829	Pivotal efficacy, therapeutic treatment	Cynomolgus monkeys	200 x LD_{50}	36 hours	36 hours
789-G923702	Efficacy, therapeutic treatment in combination with antibiotic	Cynomolgus monkeys	200 x LD_{50}	36 hours	36 hours

13.8.3.2 Time Course of Progression of Disease

The time to death in rabbits after spore challenge is reported to be approximately 2.4 days, (Zaucha et al, 1998). For non-human primates, the median time to death is 4 days (generally ranging from 3-10 days) (Friedlander et al, 1993; Kao et al, 2006; Twenhafel et al, 2007; Vasconcelos et al, 2003). In the HGS efficacy studies, the median survival time in the untreated control and placebo-treated animals ranged from 2 to 3.6 days for rabbits and 3.3 to 5.3 days for cynomolgus monkeys (Table 13-12 and Table 13-13), consistent with the values reported in the literature. Although the time to onset of disease in non-human primates and humans is similar as described above, it is more difficult to determine the time to death in humans as many of the subjects received medical intervention confounding the estimation of the time to death in untreated subjects.

Table 13-12 Results of clinical efficacy studies in the therapeutic treatment setting

Study ID	Treatment Arms	Entered	Landmark for 1° Endpoint	1° Endpoint; Survival (%)	Statistical Test: Fisher's Exact[1]	2° Endpoint Survival Time (days)	Statistical Test: Log Rank Test[2]	Comments
615-N104504	No treatment	8	Day 7	0/8 (0%)	NA	3.6	NA	-
685-G0055762	No treatment	8	Day 30	2/8 (25%)	NA	5.3	NA	-
682-G005758	Placebo	17	Day 14	0 (0.0%)	-	2.7	-	Adjustment for multiple comparisons of 1° endpoint using the Hochberg procedure
	20 mg/kg raxi	18		5 (27.8%)	0.0455	3.5	0.0181	
	40 mg/kg raxi	18		8 (44.4%)	0.0029	3.8	0.0034	
724-G005829	Placebo	12	Day 28	0 (0.0%)	-	3.3	-	Adjustment for multiple comparisons of 1° endpoint using the step-down method
	20 mg/kg raxi	14		7 (50.0%)	0.0064	-[3]	0.0029	
	40 mg/kg raxi	14		9 (64.3%)	0.0007	-[3]	0.0004	
723-G005835	Untreated	3	Day 21	0 (0)	-	2.9	-	No comparison with control was specified
	10 mg/kg levo qd x 3 d	8		7 (87.5%)	-	-[3]	-	
	25 mg/kg levo qd x 3 d	8		5 (62.5%)	-	-[3]	-	
	50 mg/kg levo qd x 3 d	8		7 (87.5%)	-	-[3]	-	
781-G923701	Placebo	12	Day 28	0 (0.0%)	-	3.3	-	Specified comparison was raxibacumab + levo vs placebo
	40 mg/kg raxi + 50 mg/kg levo qd x 3 d	20		19 (95%)	< 0.0001	-[3]	< 0.0001	
	50 mg/kg levo qd x 3 d	20		19 (95%)	< 0.0001	-[3]	< 0.0001	
789-G923702	Placebo	12	Day 28	0 (0.0%)	-	4.2	-	Specified comparison was raxibacumab + cipro vs placebo
	40 mg/kg raxi + 75 mg cipro bid x 3 d	14		12 (85.71%)	< 0.0001	-[3]	< 0.0001	
	75 mg cipro bid x 3 d	14		14 (100%)	< 0.0001	-[3]	< 0.0001	

1° primary; 2° secondary.

[1] Fisher's exact test of the intention-to-treat population.

[2] Log rank test used to compare survival time between placebo group and each of the active treatment groups.

[3] Median time to death could not be calculated because at least 50% of the animals in the group survived.

Table 13-13 Results of clinical efficacy studies in the pre/post-exposure prophylaxis setting

Study ID	Treatment Arms	Entered	Landmark for 1° Endpoint	1° Endpoint; Survival (%)	Statistical Test: Fisher's Exact[1]	2° Endpoint Survival Time (days)	Statistical Test: Log Rank Test[2]	Comments
288-HGSIRAB	Placebo	12	Day 14	0 (0.0%)	-	2	-	-
	1 mg/kg raxi	12		0 (0.0%)	1.000	3	0.0002	
	5 mg/kg raxi	12		5 (42%)	0.0373	6.5	< 0.0001	
	10 mg/kg raxi	12		10 (83%)	< 0.0001	-[3]	< 0.0001	
	20 mg/kg raxi	12		10 (83%)	< 0.0001	-[3]	< 0.0001	
	40 mg/kg raxi	12		12 (100%)	< 0.0001	-[3]	< 0.0001	
290-N005433	Placebo	10	Day 28	0 (0.0%)	-	4	-	-
	10 mg/kg raxi	10		6 (60%)	0.0108	-[3]	0.0005	
	20 mg/kg raxi	10		7 (70%)	0.0031	-[3]	0.0005	
	40 mg/kg raxi	10		9 (90%)	0.0001	-[3]	0.0001	
374-N006090	Naïve	6	Day 28	0 (0.0%)	-	3.0	-	-
	Survivors	21		21 (100%)	< 0.0001	-[3]	< 0.0001	
358-N005999	Placebo	12	Day 14	1 (8.3%)	-	3.0	-	-
	40 mg/kg raxi at 0 h	12		12 (100%)	< 0.0001	-[3]	< 0.0001	
	40 mg/kg raxi at 12 h	12		12 (100%)	< 0.0001	-[3]	< 0.0001	
	40 mg/kg raxi at 24 h	12		6 (50%)	0.0687	8.5	0.0995	
	40 mg/kg raxi at 36 h	12		5 (41.7%)	0.1550	3.5	0.0395	
371-N006101	Placebo	12	Day 14	0 (0%)	–	3.0	–	-
	5 mg/kg raxi at 24 h	12		3 (25%)	0.2174	3.5	0.0770	
	10 mg/kg raxi at 24 h	12		4 (33.3%)	0.0932	2.5	0.2408	
	20 mg/kg raxi at 24 h	12		5 (41.7%)	0.0373	6.0	0.0020	
	40 mg/kg raxi at 24 h	12		4 (33.3%)	0.0932	2.5	0.0888	
	20 mg/kg raxi at 36 h	12		0 (0%)	1.0000	3.0	0.6019	

1°, primary; 2° secondary.

[1] Fisher's exact test of the intention-to-treat population.

[2] Kaplan-Meier method used to compare survival time between placebo group and each of the active treatment groups.

[3] Median time to death could not be calculated because at least 50% of the animals in the group survived.

13.8.3.3 Manifestations of the Disease

Much of the information known about the clinical presentation and laboratory findings in human inhalational anthrax come from the detailed reports provided on the 11 cases of inhalational anthrax that occurred during the 2001 attacks. Of these 11 subjects, 5 died due to progression of the disease. The details of pathologic findings in inhalational anthrax are well described in publications describing autopsies and medical examinations done on 42 of the victims of the 1979 Sverdlovsk outbreak (Abramova et al, 1993; Grinberg et al, 2001). Reports as to the total number of victims are varied: the total number of infected subjects are reported as 68 deaths among 79 infected, while a separate report from a hospital physician recorded 358 ill with 45 dead, another recorded 48 deaths among 110 subjects. Recent analysis suggests that there may have been as many as 250 cases with 100 deaths (Inglesby et al, 2002).

The clinical presentation of inhalational anthrax has been described as a 2-stage illness. The initial symptoms most often reported are fever, nonproductive cough, headache, vomiting, weakness, chest pain, myalgia, and malaise - the clinical syndrome often resembling a viral upper respiratory tract infection (Hupert et al, 2003; Kyriacou et al, 2004). After 1 to 3 days, subjects progress to the 2nd fulminant stage of disease and exhibit the onset of fever, dyspnea, strident cough, and chills. Chest radiographs obtained during this period may demonstrate a widened mediastinum due to lymphadenopathy or hemorrhagic mediastinitis, and marked pleural effusions. Subjects then rapidly progress to respiratory failure, hypotension, shock, and in some cases death within a few hours (Dixon et al, 1999; Holty et al, 2006; Inglesby et al, 2002). Among the 11 cases identified in 2001, the precise time of exposure was known in 6 cases. The median incubation period from exposure to onset of symptoms was 4 days (range 4-6 days) (Jernigan et al, 2001). As the initial symptoms are quite nonspecific and benign, most subjects did not seek medical attention until a median of 3.5 days (range 1-7 days) after the onset of illness (Jernigan et al, 2001). In all 11 cases of inhalational anthrax seen during the 2001 attacks, malaise and fever were the presenting symptoms. All of the subjects had abnormal chest radiograph findings within several hours of presentation – the majority of findings were mediastinal widening, pulmonary infiltrates or consolidation, and massive pleural effusion (Inglesby et al, 2002). Many of the 2001 cases involved rapid progression of disease, requiring transfer to the intensive care unit with mechanical ventilation and hemodynamic support.

Laboratory findings of leukocytosis, elevated transaminases, and hypoxemia were commonly seen during the course of illness (Barakat et al, 2002;Jernigan et al, 2002). The diagnosis of inhalational anthrax infection was made by positive identification of the organism in cultures obtained from blood, cerebrospinal fluid, or pleural fluid in 8/11 cases (Jernigan et al, 2002). Blood cultures demonstrated bacterial growth after 6 to 24 hours of incubation with preliminary identification of Bacillus species (Inglesby et al, 2002). Blood cultures were sterilized after only 1 or 2 doses of antibiotics. Alternative supportive tests used in the 11 cases included demonstration of *B. anthracis* by immunohistochemical staining or polymerase chain reaction (PCR) testing of blood, pleural fluid, or tissue samples obtained from bronchial or pleural biopsy. There is currently no published information available about determinations of PA measurements in samples obtained from the 11 subjects. Measurement

of anti-PA antibodies in survivors of suspected anthrax disease (6 with inhalation anthrax and 11 with cutaneous anthrax), showed that all 6 subjects with inhalation and 10/11 subjects with cutaneous anthrax developed an anti-PA response within 11 days of the onset of symptoms (or 15 days of likely exposure) (Quinn et al, 2004).

Autopsies from the 42 subjects examined after the Sverdlosk outbreak indicate that the most prominent and consistent lesions in subjects with inhalational anthrax are hemorrhagic mediastinal and thoracic lymph nodes. Many subjects exhibited a focal hemorrhagic necrotizing pneumonitis, possibly indicating the portal of infection. Evidence of hematogenous spread of infection was seen in submucosal lesions in the gastrointestinal tract (stomach, small intestine, and colon) and hemorrhagic lesions in the leptomeninges (Abramova et al, 1993). Gram-positive organisms were noted in most affected organs, including the lungs, lymph nodes, intestinal submucosa, leptomeninges, heart, spleen liver, brain, and less commonly in the kidneys and adrenals. The most severe and advanced lesions in each case were in the mediastinal lymph nodes, suggesting that this involvement was the earliest in the course of the disease. Examination of the lung tissues revealed acute bronchopneumonia in nearly half of the subjects. Hemorrhage and vasculitis were frequently seen in the meninges and cerebral parenchyma (Grinberg et al, 2001).

The pathology of the inhalational form of the disease in non-human primates is remarkably similar to that observed in humans (Fritz et al, 1995; Twenhafel et al, 2007; Vasconcelos et al, 2003). The most significant pathology is edema, hemorrhage and necrosis with leukocyte infiltration in various tissues including mesenteric and tracheobronchial lymph nodes, meninges, lungs and small intestine (Fritz et al, 1995). However, splenic pathology was different between humans and non-human primates, since all of the non-human primate studies demonstrated splenomegaly. In addition, the severity of the neutrophilic inflammation and the fibrin exudation appears to be greater in the spleens of non-human primates. Mediastinal enlargement due to different degrees of edema and hemorrhage is a consistent finding across chimpanzees, rhesus macaques, and cynomolgus monkeys (Vasconcelos et al, 2003). Henderson and his colleagues provided data suggesting that inhaled *B. anthracis* spores in the lung alveoli of rhesus macaques initiate infection and are phagocytosed by macrophages which either remain in the primary infection site or migrate to regional lymph nodes (Henderson et al, 1956).

After inhalation of a lethal dose of *B. anthracis* spores, death occurs in non-human primates typically between 2 and 10 days with a median of approximately 4 days. The LD_{50} in rhesus macaques has been shown to be 5.5×10^4 spores, while it is 4.13×10^3 spores in the cynomolgus macaque (Vasconcelos et al, 2003). Clinical signs of the infection include fever, lethargy, weakness and anorexia manifested generally 1 to 4 days preceding death. Bacteremia is also present for 2 days before death. Over the past decades, extensive research on non-human primates for inhalational anthrax has made the non-human primate model a well-suited system to evaluate new therapies for inhalational anthrax. The non-human primate model was used to establish the efficacy of ciprofloxacin, doxycycline and levofloxacin in post-exposure prophylaxis setting (Friedlander et al, 1993; Kao et al, 2006; Meyerhoff et al, 2004)

Several rabbit species have been used as models to study inhalational anthrax infection; the most widely employed being the NZW rabbit. Lesions observed in NZW rabbits were comparable with those of inhalational anthrax in humans and rhesus monkeys (Zaucha et al, 1998). The most significant pathological changes occurred in the lymph nodes, spleen, lungs, adrenal glands, and gastrointestinal tracts. Notable differences are lack of leukocyte infiltration in brain and meningeal lesions, the relatively mild mediastinal lesions, and a lower incidence of anthrax-related pneumonia compared with humans. This discrepancy may be attributed to the rapid progression of disease in this species, which presumably limits development of leukocyte infiltrates in response to hemorrhage and necrosis.

The mean survival time of rabbits with inhalational anthrax is 2.4 days and clinical signs are not manifested until within 24 hours of death. The calculated LD_{50} for *B. anthracis* Ames strain in rabbits is 1.1 x 10^5 colony forming units (CFU) which is higher than the LD_{50} in non-human primates. Although the rapid fatal course of inhalation anthrax could be considered disadvantageous, the rabbit model has several attractive features compared with non-human primate models: rabbits are considered lower phylogenic species and are easier to obtain and safer to house and handle than non-human primates (Phipps et al, 2004).

Other hallmarks of anthrax disease progression, including toxemia, bacteremia, temperature increase, changes in hematology and indicators of inflammation (eg, CRP) are described in detail in Appendix 13.9 which discusses the model characterization studies performed in rabbits and monkeys (Studies 615-N104504 and 685-G005762) to identify an appropriate trigger for therapeutic intervention. Not only does anthrax disease progression have similar features in the rabbit and monkey, it mirrors that of the human disease. Histologic changes observed in the spore-challenged rabbits and monkeys are discussed by study in Appendix 13.10 and summarized in Appendix 13.13. Both the parameters of disease progression and the pathophysiology of the disease are similar in rabbits, monkeys and humans.

13.8.4 Trigger for Intervention

To support an indication in therapeutic treatment, raxibacumab was to be administered to animals that were symptomatic for anthrax disease. Model characterization studies were conducted in both species (615-N104504 and 685-G0055762) to identify clinical or laboratory parameters that could be used as triggers for therapeutic intervention and the selection of detectable serum PA or increased temperature in rabbits or detectable serum PA in monkeys were identified as appropriate triggers. Serum PA measurement had the advantage of providing a sensitive and anthrax-specific result within 1 to 2 hours of sample collection, and was determined to be one of the 1st indicators of systemic anthrax infection. Bacteremia measured by culture or PCR had an onset contemporaneous with serum PA and is also sensitive and anthrax-specific. However, culture results cannot be obtained in less than 24 to 48 hours and PCR requires equipment and preparation unsuited to its use in a BL3 facility. Moreover, PCR results may not be available for several hours after sampling. Temperature increase in rabbits slightly lagged serum PA and bacteremia in time of onset, but had the advantage of a real-time measurement. Consequently, temperature increase was used along

with serum PA (whichever appeared first) in the rabbit studies. In monkeys, the strong diurnal temperature rhythms confound the use of increased temperature. Other parameters such as hematology changes and chemistry markers, for example C-reactive protein (CRP), are also hallmarks of systemic infection, but are lagging, non-specific indicators compared with serum PA and the results are available no more rapidly. See Appendix 13.9 for additional details of the model characterization studies.

13.8.5 Characterization of Medical Intervention

13.8.5.1 Product Class

Raxibacumab is a fully human monoclonal antibody which specifically binds PA of *B. anthracis*. Clinical safety studies in humans and nonclinical safety studies in rabbits and monkeys have shown raxibacumab to be safe and well tolerated. Fully human and humanized monoclonal antibodies are increasingly used in the treatment of human disease, including oncology, immunology, and infectious disease.

Use of human antibodies in animal models may present problems of immunogenicity as the human protein is foreign to other species. In rabbits that received multiple injections as part of the embryo-fetal toxicology study (Study 6962-173), anti-raxibacumab antibodies were detected in 4 of 20 animals, but exposure to raxibacumab was maintained even in these animals with no indication of any allergic or inflammatory reaction. In Study 781-G923701, anti-raxibacumab antibodies emerged in a majority of rabbits in the active treatment arms by Day 28, however, survival was very high in these groups, indicating that the anti-raxibacumab antibodies did not interfere with efficacy. In contrast, in monkeys receiving single or multiple administrations of raxibacumab at and above the recommended clinical dose of 40 mg/kg, there was no detectable anti-raxibacumab response. Consequently, rabbits and monkeys are suitable species in which to study the efficacy of a human monoclonal antibody.

Because the target for raxibacumab, *B. anthracis* PA, is not an endogenous protein, there is little or no cross reactivity with other tissues in healthy humans, rabbits or monkeys and no evidence of exaggerated pharmacology in any of the 3 species in toxicology or human safety studies.

13.8.5.2 Mechanism of Action

Anthrax disease is caused by infection with the gram-positive spore-forming bacterium, *B. anthracis*, which produces anthrax toxins resulting in cellular intoxication and death, particularly in macrophages. The mechanism of action of anthrax toxin has been well characterized and reported in the literature. Inhibition of PA binding to its cellular receptors can abrogate the downstream toxin-mediated deleterious effects of the anthrax toxins. Accordingly, the association between PA and ATRs represents a critical molecular junction in the progression of anthrax disease, and inhibition of this interaction is a viable clinical strategy against anthrax toxicity. Indeed, interference with the PA-ATR association abrogates anthrax toxin associated pathogenesis as previously described in the literature in vitro (Little et al, 1990; Maynard et al, 2002) and in vivo (Kobiler et al, 2002;

Maynard et al, 2002). Raxibacumab inhibits the anthrax toxin-mediated effects by preventing PA from binding to its receptors.

13.8.5.3 In Vitro Activity

A variety of in vitro studies were undertaken to evaluate the pharmacologic properties of raxibacumab based on the reported mechanism of action of anthrax toxins. These studies are described in Appendix 13.1.

13.8.5.4 Activity in Disease with Common Pathophysiology

Based on the in vitro data, pharmacology studies (Appendix 13.2) were extended to an in vivo anthrax model in rats using anthrax lethal toxin injection where death results within 90 minutes (Cui et al, 2004). The lethal toxin injection replicates the downstream effects of anthrax inhalation, but does not use inhalation of spores. Lethal toxin is the combination of the receptor-binding component, PA, and the metalloprotease, LF, of *B. anthracis* (Friedlander et al, 2001). Three in vivo studies in rats evaluated raxibacumab administered via IV, SC, or intramuscularly (IM) as a pre-exposure prophylaxis treatment from 60 minutes up to 8 weeks prior to lethal toxin exposure. Collectively, these studies provided proof of concept that a single dose of raxibacumab administered prophylactically by a variety of routes can provide full protection from the lethal effects of anthrax toxin and afford 100% survival when administered up to 3 weeks prior to exposure.

Additional in vivo studies were conducted in a 24-hour continuous lethal toxin infusion rat model, in which death is delayed until approximately 9 to 21 hours following lethal toxin infusion with an overall 7-day mortality rate of 53% (Cui et al, 2004). The longer time to death in this model allowed investigation of raxibacumab treatment in the post-exposure treatment setting, where rats begin to develop hemodynamic instability approximately 6 hours following lethal toxin initiation (Cui et al, 2005). Raxibacumab treatment up to 6 hours following lethal toxin initiation resulted in significant improvements in hemodynamic effects and survival despite the presence of established hemodynamic instability and developing shock. Treatment with raxibacumab as late as 12 hours after initiation of lethal toxin also improved hemodynamic effects and survival at a level approaching significance.

13.8.5.5 Pharmacokinetics in Affected Animals/Humans

The PK of raxibacumab in healthy rabbits, cynomolgus monkeys and humans has been well established and the PK in each of these species is described in the main body of the briefing document.

Raxibacumab PK in humans, rabbits, and monkeys are linear, predictable, and allow extrapolation between species. Peak raxibacumab concentrations (C_{max}) following a 40 mg/kg IV administration have been remarkably consistent across healthy humans, and healthy and anthrax-infected rabbits and monkeys as shown in Table 13-14. The results for C_{max} across the human studies are in good agreement with those from the healthy rabbit and monkey studies.

In all 3 species, the initial volume of distribution approximates the plasma volume and the steady state volume of distribution indicates that raxibacumab subsequently distributes to tissues. Clearance of raxibacumab is more rapid in rabbits and monkeys than humans, but raxibacumab exposure is sustained in all 3 species.

Table 13-14 Peak raxibacumab concentrations (C_{max}) following a 40 mg/kg IV dose

Species	Study #/Report	Peak Concentration for 40 mg/kg Dose (µg/mL)
Human	Phase 1 (PAM-NH-01)	1042 ± 88
	Raxibacumab/ciprofloxacin (HGS1021-C1064 Group 1[1])	1103 ± 225
	Raxibacumab (HGS1021-C1064 Group 2)	988 ± 220
	Raxibacumab/ciprofloxacin (HGS1021-C1064 Group 3[2])	1048 ± 180
	Reinjection (HGS1021-C1069)	979 ± 148
	Population PK analysis including HGS1021-C1063 (HGS1021-POP01)	960 ± 164
Rabbit	Healthy rabbit (AB50409.INF.0.016)	1104 ± 75.6[3]
	Embryo-fetal toxicology (6962-173/AB50409.INF.0.038)	1022 ± 46
	Pre-exposure prophylaxis (288-HGSIRAB/AB50409.INF.0.027)	787 ± 207
	Pivotal efficacy study (682-G005758/AB50409.INF.0.036)	918 ± 124
	Raxibacumab/levofloxacin (781-G923701/AB50409.INF.0.043)	928[4]
Monkey	Healthy monkey (AB50409.INF.0.017)	1046 ± 12[3]
	Toxicology (6962-140/AB50409.INF.0.034)	1089 ± 113
	Confirmatory efficacy (724-G005829/AB50409.INF.0.040)	990 ± 170
	Raxibacumab/ciprofloxacin (789-G923702/AB50409.INF.0.042)	1060[4]

[1] Raxibacumab administered in combination with PO ciprofloxacin.

[2] Raxibacumab administered in combination with IV and PO ciprofloxacin.

[3] Extrapolated from 10 mg/kg IV administration.

[4] Based on population PK parameters.

13.8.5.6 Pharmacokinetics/Pharmacodynamics in Unaffected Animals/Humans

The PK in anthrax-challenged rabbits and monkeys are described in the main body of the briefing document. No studies have been conducted with raxibacumab in humans with anthrax disease as it is unethical to challenge humans with anthrax spores. As shown in Table 13-14, the peak raxibacumab concentrations are highly similar between healthy and anthrax-challenged animals, as well as among species. Although peak concentrations are the same in healthy and diseased animals, in anthrax spore-challenged rabbits and monkeys, clearance is higher and the area under of curve of raxibacumab (AUC) is lower than for healthy animals. These data suggest that raxibacumab peak exposure and volume of distribution are about the same in healthy and spore-challenged animals, but that systemic disease and PA toxemia increase the apparent clearance of raxibacumab.

The kinetics of serum PA in rabbits and monkeys were modeled using the data from spore-challenged untreated and placebo-control animals. Serum PA concentration-time data in rabbits and monkeys were best described using a diauxic growth model. The model consists of 2 main phases; in the 1st phase, PA concentrations are consistent with a Gompertz model, whereas the 2nd phase consists of a 2nd period of exponential growth, which starts following a lag time. Hence, the resulting serum PA concentration-time profile has a lag period-rise-plateau-rise shape. PA kinetics in rabbits are characterized by an earlier onset of the both rising portions of the profile, a more rapid rate of increase in the 1st rising phase, and a slightly slower rate of increase in the 2nd rising phase, compared with monkeys. The similarity of covariates for rabbits and monkeys (time of 1st detectable bacteremia or survival time) reflect the disease process: the 1st phase lag time for the PA profile is associated with time to 1st bacteremia, which is expected given that the appearance of circulating bacteria would be contemporaneous with the appearance of circulating PA; and longer survival times are associated with lower plateau concentrations as well as slower rates of increase during the 2nd rising phase, which is also plausible as longer time to death is likely to be associated with lower and less rapid increase in PA exposure.

The predicted percent of PA bound by raxibacumab was calculated, based on the dissociation equilibrium constant (K_d) determined in vitro, as well as the lower 90% prediction interval bound for the predicted human serum raxibacumab concentration-time profile (thus ensuring 95% of human subjects would have concentrations equal to or greater than that value). On that basis, it was determined that a single 40 mg/kg IV raxibacumab dose in humans is adequate to bind at least 99.7% of serum PA for up to 28 days after administration, in at least 95% of subjects.

Overall, based on raxibacumab PK in rabbits, monkeys, and humans, as well as serum PA in *B. anthracis* spore-challenged rabbits and monkeys, it appears that a single 40 mg/kg raxibacumab dose in humans should have efficacy for the treatment of inhalation anthrax that would be comparable to that observed in the nonclinical therapeutic efficacy studies. Evaluation of a 20 mg/kg dose for humans indicates that it would likely be inferior to the 40 mg/kg dose, in that a 20 mg/kg dose would not provide exposures greater than or equal to those proven to be associated with survival in nonclinical studies for all subjects, and would not provide sufficient duration of protection for innate immunity to develop. In contrast, a 40 mg/kg dose to humans should result in virtually all subjects attaining exposures associated with survival in the nonclinical studies, with duration of protective levels of at least 28 days for the majority of subjects, allowing the development of innate immunity. Hence, a human raxibacumab dose of 40 mg/kg is justified.

13.8.5.7 PK Interactions with Medical Products Likely to be Used Concomitantly

Because the current standard therapy for inhalation anthrax includes post-exposure prophylaxis with antimicrobials, the efficacy of antibiotics administered concomitantly with raxibacumab was evaluated in rabbits (Study 781-G923701) and monkeys (Study 789-G923702) in the setting of therapeutic treatment with symptomatic anthrax disease. In these studies, raxibacumab did not alter the efficacy of levofloxacin or

ciprofloxacin administered at doses providing exposures reflective of those obtained at recommended doses in humans, nor did the concomitant administration of levofloxacin or ciprofloxacin with raxibacumab alter the PK of any of the products. The PK of raxibacumab and ciprofloxacin (IV and PO) were also evaluated in healthy volunteers and neither drug altered the PK of the other compound (HGS1021-C1064).

The effect of diphenhydramine on raxibacumab PK was evaluated because the recommended dosing instructions for raxibacumab include prophylactic administration of diphenhydramine prior to raxibacumab administration. Comparative PK analyses indicate that raxibacumab exposure was not altered in subjects administered diphenhydramine, relative to those who did not receive diphenhydramine and diphenhydramine use was not a covariate for raxibacumab PK (HGS1021-C1064).

Since raxibacumab is fully human IgG, it is reasonable to expect that it would have no effect on the PK or pharmacodynamics (PD) of other medical products likely to be used in the treatment of a subject with signs and symptoms of inhalation anthrax, such as anti-pyretics, blood pressure modifiers, or fluids.

13.8.5.8 Synergy or Antagonism of Medical Products Likely to be used in Combination

Because raxibacumab is likely to be used concomitantly with antimicrobials, the efficacy of levofloxacin and ciprofloxacin in combination with raxibacumab was evaluated in rabbits (Study 781-G923701) and monkeys (Study 789-G923702), respectively. The antimicrobial doses (50 mg/kg levofloxacin and 75 mg ciprofloxacin) were chosen to provide similar exposure in animals to the exposure achieved in humans at the recommended doses of these antibiotics. These studies were designed to allow observation of a negative impact of raxibacumab on the antimicrobials, not to demonstrate its potential for added benefit. In both studies, mortality in the control group was high (0% survival in both studies) and the antibiotics alone provided statistically significant protection against mortality (95% survival in rabbits treated with levofloxacin and 100% in monkeys treated with ciprofloxacin) and concomitant administration of raxibacumab did not significantly alter the efficacy or PK of the antimicrobials.

While it is of interest to know if raxibacumab provides added benefit beyond that afforded by antimicrobials alone, it is not feasible to evaluate the additive or synergistic effect of raxibacumab on antimicrobials when the antimicrobials are administered at a dose which provides equivalent exposure to that of the recommended human dose. Given the high survival rate in antibiotic-treated rabbits and monkeys, a study can not be adequately powered to statistically evaluate a survival benefit of the raxibacumab/antimicrobial combination arm over antimicrobial alone due to limitations on the number of animals that can ethically and logistically be included in a study. For example, the sample size to yield 80% power at the 5% significance level to detect a 20% greater survival benefit of raxibacumab/antimicrobial (83%) vs antimicrobial alone (63%) would require 75 monkeys per group. With survival rates approaching 100% in animals given human-equivalent doses, the number of animals needed per group would be even larger.

While it may be possible to develop an animal model in which the timing and or dose of antimicrobials are selected to reduce the survival rates to a level that allows an adequately powered study to be conducted to test superiority of raxibacumab/antimicrobial dosing over antimicrobials alone, that model has not yet been reduced to practice and would likely require lower antimicrobial doses than the recommended human-equivalent doses. A recent publication by Vietri et al (2009), suggests that early and truncated dosing of antibiotics at the time of spore challenge is less effective (20% survival rate) than therapeutic treatment of animals after they have become bacteremic (70% survival rate). This type of study design could form the basis of a test of added benefit in the setting of prophylactic antibiotics and therapeutic raxibacumab administration, but not for antimicrobials and raxibacumab administered concomitantly as therapeutic treatment.

Further to correspondence from the FDA, the superiority of raxibacumab over placebo or the combination of raxibacumab/antimicrobial over antimicrobial alone can be the basis for licensure. The superiority of raxibacumab over placebo has been demonstrated in rabbits and monkeys as the basis for licensure in this application. The raxibacumab/antimicrobial studies have demonstrated that raxibacumab does not interfere with the efficacy or PK of levofloxacin or ciprofloxacin.

13.8.6 Design Considerations for Efficacy Studies

Because the pivotal efficacy studies provide the primary support of efficacy for licensure, they were designed and performed in a manner consistent with human clinical efficacy trials. Protocols and Analytical Plans prespecified the study design and data analysis. The studies were powered to provide a statistically significant result if a clinically important increase in survival was observed, and appropriate statistical adjustments were made for multiple comparisons of the primary endpoint for the 2 raxibacumab doses in the efficacy studies. Particular considerations for endpoints, timing and route of administration and dosing regimen are summarized below and discussed in greater detail in the referenced sections.

13.8.6.1 Endpoints

The primary endpoint in all of the efficacy studies was survival rate at a landmark time. The secondary endpoint of the efficacy studies was survival time (ie, time to death after spore challenge).

The primary endpoint, survival rate, is relevant to the outcome of human disease which is highly lethal, and the secondary endpoint of survival is of importance because longer survival times permit a larger window in which to provide medical intervention to resolve the infection and to allow development of innate immunity against PA

13.8.6.2 Timing of Intervention

Model characterization studies (615-N104504 and 685-G005762) were performed to identify appropriate triggers for intervention. First detectable serum PA was chosen as an appropriate trigger in rabbits and monkeys, while sustained temperature was also a trigger in rabbits, but not monkeys due to their strong diurnal temperature rhythms. The presence of bacteremia

and/or toxemia at the time of treatment was confirmed and the vast majority of animals were bacteremic and/or toxemic at the time of treatment. Moreover, the statistically significant survival benefit for raxibacumab-treated animals was maintained in the subgroups of animals that were confirmed to be bacteremic and/or toxemic at the time of treatment.

13.8.6.3 Route of Administration

The route of raxibacumab administration was IV in all of the animal therapeutic efficacy studies. IV administration of raxibacumab is consistent with its use in a clinical setting for treatment of a subject with signs and symptoms of inhalation anthrax. Treatment for inhalation anthrax in this setting would include other IV medications (eg, anti-pyretics, blood pressure modifiers, fluids, and antibiotics).

The efficacy of raxibacumab administered by the SC route was evaluated in the pre-exposure prophylaxis setting in rabbits and monkeys, in which SC administration of 5 to 40 mg/kg raxibacumab significantly increased survival rates, approaching 100%. However, the dose recommended for therapeutic treatment, 40 mg/kg, is too large to provide by SC administration given the current formulation for raxibacumab. Also the delayed attainment of high raxibacumab concentrations for SC dosing (t_{max} ~ 2 days) is unsuitable for therapeutic intervention, where rapid disease progression and death can occur.

Intramuscular injection has been evaluated in humans with well-behaved PK and good tolerability (PAM-NH-01). However, the IM doses tested were less than those demonstrated to be effective as therapeutic treatment. Like for the SC route, a more concentrated solution and earlier invention times may permit the use of raxibacumab for a pre- or post-exposure prophylaxis, but these are not the indications being sought in the current application.

The antimicrobials evaluated in the animal studies were administered by oral gavage, a route consistent with oral use. In the human safety and PK study, ciprofloxacin was administered both PO and IV, to reflect possible use scenarios in the clinic. Raxibacumab was compatible with concomitant ciprofloxacin administration by both routes.

13.8.6.4 Dosing Regimen

Raxibacumab is to be provided as a single IV administration. This is the dosing regimen used in all of the therapeutic efficacy studies to support licensure and the route of administration in the human safety studies. Repeat raxibacumab administration either after 14 days to reflect a 2nd injection in the event of protracted disease (HGS1021-C1063) or after 4 or more months to reflect administration after washout (HGS1021-C12069), demonstrated that repeat injection is safe and well tolerated with predictable PK and no immunogenicity.

13.8.7 Available Safety Information

The safety, tolerability and immunogenicity of raxibacumab have been studied in more than 400 healthy volunteers, of whom 332 have received 40 mg/kg raxibacumab manufactured and formulated as intended for licensure. Per agreement with FDA, a safety database of 300 subjects treated with the formulation and manufacturing process proposed for licensure was required to support an indication in therapeutic treatment of inhalation anthrax. In the

safety studies, raxibacumab was safe and well tolerated with a rate of adverse events (AEs) no different from that of placebo-treated subjects. There were no deaths among the raxibacumab treated subjects and a single serious adverse event (SAE) of cholecystitis, which the investigator deemed most likely caused by the subject's underlying condition.

Premedication with diphenhydramine was introduced during Study HGS1021-C1064 and has been successful in that and subsequent studies in preventing the mild to moderate rashes observed in some of the 1st subjects in the study who were not premedicated. The rashes were generally mild to moderate and resolved without medical treatment or with the administration of diphenhydramine. The rashes were not associated with anaphylactoid reactions and there was no evidence of immunogencity after raxibacumab in any study which would suggest an increased risk of infusion reactions upon readministration.

13.8.8 Limitations of the Animal Models

Despite the similar pathology of inhalation anthrax in rabbits, non-human primates and humans, there are differences among the species and limitations of the models compared with the human clinical setting.

- The more rapid time course of disease in the rabbits limits the window of intervention between onset of symptoms and death, resulting in higher mortality in the rabbits than in non-human primates or humans.
- The anthrax challenge in the animal models is controlled with respect to number of spores and intensity and duration of delivery. The duration, intensity and total anthrax exposure in a bioterror attack is unknown, so the time course of disease and the efficacy of raxibacumab in humans may be different than that observed in the animal models, although the simultaneous germination of very high spore challenges (ie, 200 x LD_{50}) should provide a rigorous test of raxibacumab efficacy, as evidenced by the high mortality rate in both rabbits and monkeys.
- The rabbits and monkeys in the model experiments receive no supportive care (ie, lung drainage, fluids, blood pressure modifying agents, anti-pyretics, anti-emetics or pain medication) which would comprise standard of care for humans with inhalation anthrax. This supportive care is likely to increase the time from onset of symptoms to death compared with that observed in the animal models.
- Raxibacumab has been administered either as pre-exposure prophylaxis, post-exposure intervention or as therapeutic treatment at the time of the onset of systemic disease (ie, temperature increase or detectable serum PA). In these settings raxibacumab provides a statistically significant survival benefit compared with placebo. The efficacy of raxibacumab administered at longer times after the onset of clinical symptoms has not been studied and it is likely that there is a point at which the damage from bacteria and their toxins cannot be reversed and raxibacumab (and/or antimicrobials) will not be effective. How long raxibacumab administration can be delayed after the onset of symptoms and still provide a survival benefit is unknown.

With respect to concomitant administration with antimicrobials, the doses of antimicrobials (levofloxacin and ciprofloxacin) were chosen to provide an equivalent maximum concentration to those achieved with the recommended human doses, and the antimicrobials were administered concomitantly with raxibacumab at the onset of systemic disease per request by the FDA. This study design presents some limitations relative to the human clinical setting.

- The doses and regimens used in animal studies were highly protective against mortality alone and in combination with raxibacumab (> 85%). In contrast, in the anthrax attacks in 2001, the survival rate in humans administered multiple antibiotics was 55%.
  - This high survival rate makes it unfeasible to demonstrate added benefit of raxibacumab in combination with antimicrobials and the magnitude of additional benefit, if any, provided by raxibacumab when used with recommended antimicrobial doses as therapeutic treatment in humans is unknown.
- Raxibacumab has only been studied with concomitant administration with antimicrobials at the onset of disease. There are no efficacy studies evaluating the efficacy of antimicrobials and raxibacumab when the antimicrobials are administered prophylactically and raxibacumab is administered therapeutically. Because raxibacumab does not alter antimicrobial efficacy or PK, the efficacy of this regimen should be at least that of the antibiotics alone and would provide anti-PA activity at the time PA is present.
- The standard of care for inhalation anthrax includes administration of multiple antimicrobials, as well as other medications for supportive care. Raxibacumab has only been studied in combination with a single antibiotic and not in subjects receiving other medications for the treatment of inhalation anthrax. However, because raxibacumab is fully human IgG, it is reasonable to expect that it would have no effect on the PK or PD of other medical products likely to be used in the treatment of a subject with signs and symptoms of inhalation anthrax, such as anti-pyretics, blood pressure modifiers, or fluids.

13.9 Appendix 9: Model Chacterization Studies

13.9.1 Characterization of Rabbit Model (Study 615-N104504)

This non-GLP study, "Exploratory Study to Evaluate Markers of Disease Course of *Bacillus anthracis* in NZW Rabbits," examined the time to onset of abnormal values in physiological (eg, temperature, bacteremia, serum PA, and hematology) and clinical signs (eg, moribund, respiratory distress, appetite, activity, and seizures) to obtain an understanding of disease progression post-anthrax spore exposure, and to identify a parameter(s) that indicates an optimal window of time for therapeutic intervention.

Study Design and Analysis

Eight NZW rabbits (4 male and 4 female) were exposed to a target of 200 x LD_{50} *B. anthracis* spores (Ames strain). Because this was a natural disease history study, no treatment was administered. The parameters to be measured included clinical observations, temperature, hematology, CRP, serum PA, bacteremia by culture and PCR, and minimum inhibitory concentration (MIC) for levofloxacin and ciprofloxacin.

The primary analysis of this study was examination of the relationship between an animal's survival time vs time to onset of clinical parameters indicative of anthrax infection including bacteremia (by culture and PCR), detectable serum PA, and clinically significant increase in body temperature.

Descriptive statistics were used to summarize dose of *B. anthracis* spores, survival time, time to onset of bacteremia (by culture and by PCR), time to detectable serum PA, and time to clinically significant increase in body temperature. Spearman correlation coefficients were used to assess the correlation between survival time vs time to onset of bacteremia (by culture and by PCR), time to detectable serum PA, and time to clinically significant increase in body temperature.

Results

The rabbits were challenged with a minimum of 93 x LD_{50} to a maximum of 278 x LD_{50} dose of *B. anthracis* (Ames strain) spores. The average exposure for all challenged rabbits on study was 178 ± 57 x LD_{50}.

Seven of 8 rabbits died within the 7-day post-exposure monitoring period; the 8th animal was euthanized at the end of the prespecified 7-day monitoring period. The earliest time of death was 48 hours and the longest time was greater than 168 hours for the animal that was euthanized at study end. There was no statistically significant correlation between the extent of spore exposure and the time to death ($p = 0.3013$).

All 8 rabbits had serum PA detected after spore challenge. Three had PA detected at 24 hours post spore challenge, the earliest time point at which PA concentrations were measured (Figure 13-11). The median time to 1st detection of serum PA was 30 hours. The mean serum PA concentration-time profile shows an initial rise in serum PA up to about

36 hours post challenge, followed by a period during which the concentrations plateau, followed by a 2nd period of rising concentrations.

All rabbits became bacteremic by culture during the study. Bacteremia by culture was observed as early as 20 hours after spore challenge and half of the rabbits were bacteremic at or before 24 hours after spore challenge (Figure 13-11). The median time to bacteremia by culture was 26 hours. All rabbits became bacteremic by PCR (using the pagA primer) during the study. Bacteremia by PCR was observed as early as 20 hours after spore challenge and 5 out these 8 rabbits became bacteremic at or before 28 hours after spore challenge. The median time to bacteremia by PCR was 28 hours.

All rabbits had clinically significant temperature increases (ie, ≥ 2°F) after spore challenge. The time to clinically significant temperature increase ranged from 26 to 78 hours with a median time to temperature increase of 32 hours after spore challenge. The observation of 1st temperature rise occurred at or shortly after detection of bacteremia and serum PA and was contemporaneous with changes in WBC (Figure 13-11).

Of the hematology parameters, WBC was the most indicative of disease progression. WBC was relatively stable in all of the animals through 20 hours post spore challenge. From 24-36 hours, most animals exhibited sharp declines in WBC. These decreases were followed over the next 2 days by increases in WBC to levels at or exceeding baseline values (Figure 13-11).

Elevations in CRP were observed in most of the animals that died while on study and the increases in CRP generally trailed the onset of bacteremia and temperature increase (Figure 13-11).

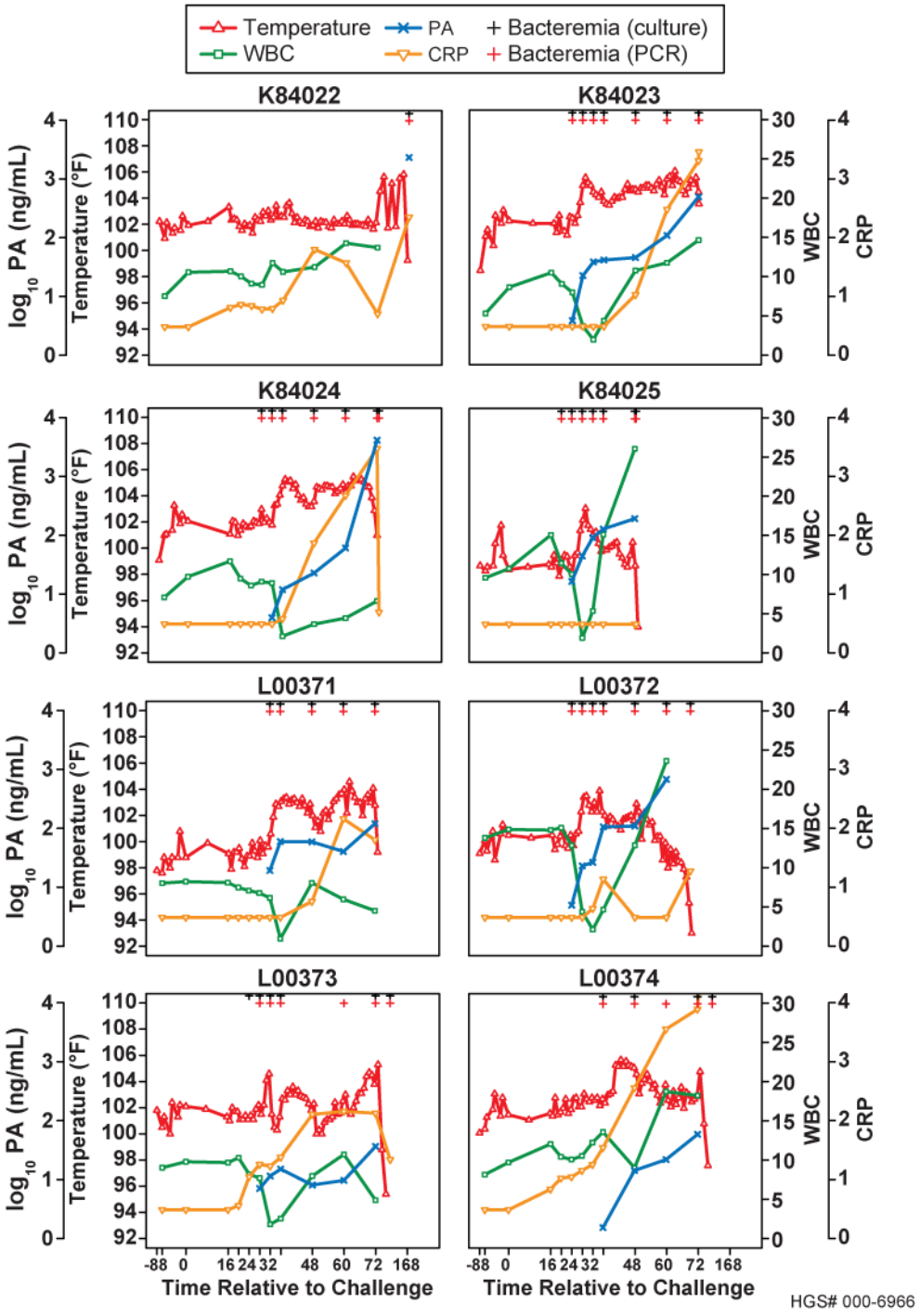

Figure 13-11 Time course of clinical parameters in rabbit model of inhalation anthrax (615-N104504)

Illustration of key clinical parameters in individual rabbits following anthrax spore challenge. Upper 2 panels, males; Lower 2 panels, females.

Survival time was strongly correlated with the time to detection of PA (r = 0.896, p = 0.0026) indicating that the appearance of serum PA was a good predictor of mortality. Detection of bacteremia measured by culture was consistent with bacteremia detection by PCR, and survival time was strongly correlated with time to bacteremia by culture (r = 0.854, p = 0.0070) and PCR (r = 0.952, p = 0.0003). Survival time was also strongly correlated with time to a significant increase in body temperature (r = 0.833, p = 0.0102), although less so than with serum PA or bacteremia. Time to 1st detected serum PA was also highly correlated with 1st detected bacteremia (by either method) and with time to 1st temperature rise.

The MIC for levofloxacin ranged from 0.50 to 1.0 µg/mL and the MIC for ciprofloxacin ranged from 0.125 to 0.5 µg/mL and did not differ for the bacteria isolated at the end of the study from the starting control bacteria.

Serum PA and bacteremia by culture or PCR appear to be the 1st indicators of anthrax disease, followed shortly thereafter by temperature rise and changes in WBC (Figure 13-12), and subsequently by increases in CRP. This suggests that the appearance of PA in serum is coincident with the appearance of bacteremia, and is an antecedent to increased temperature. The sequence of these events was similar in all animals, except Animal K84022 who survived to study end (Day 7). That animal did, however, present with bacteremia by culture and PCR, measurable serum PA and temperature instability (increased temperature followed by decreased temperature) at the time it was euthanized (Figure 13-11).

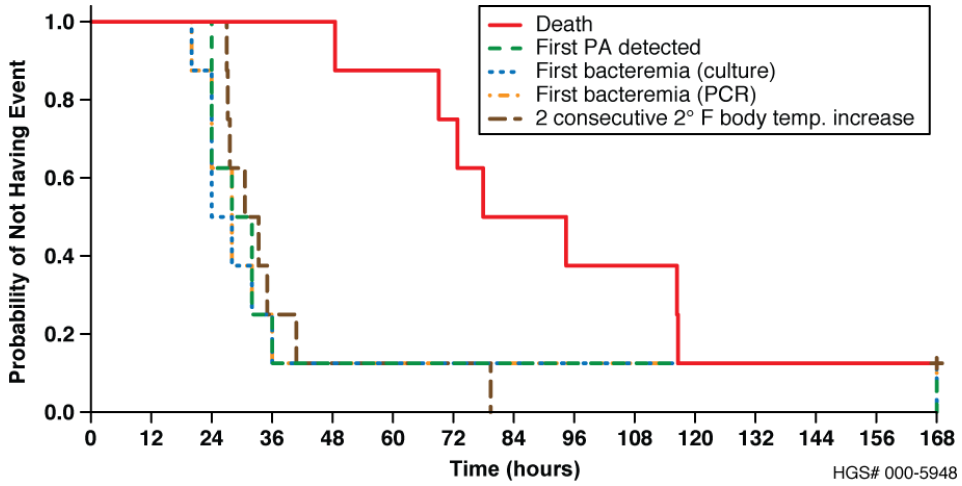

Figure 13-12 Time to event for clinical parameters in anthrax-challenged rabbits (615-N104504)

13.9.2 Characterization of Cynomolgus Monkey Model (Study 685-G005762)

This non-GLP study, "Natural History Study to Evaluate Criteria for Evidence of Illness due to Inhalation Anthrax in Cynomolgus Macaques," examined time to onset of abnormal values in physiological and clinical signs to obtain a better understanding of disease progression in

cynomolgus monkeys post-anthrax spore exposure and to identify a parameter(s) that indicates an optimal window of time for therapeutic intervention.

Study Design and Analysis

Eight cynomolgus macaques (7 male, 1 female) were exposed to a target of 200 x LD_{50} *B. anthracis* spores (Ames strain). Because this was a natural disease history study, no treatment was administered. The parameters to be measured included clinical observations, telemetry, hematology, CRP, serum PA, bacteremia by culture and PCR, and toxin neutralization activity (TNA).

The primary analysis of this study was examination of the relationship between survival time and time to onset of clinical parameters indicative of anthrax infection including bacteremia (by culture and PCR), detectable serum PA, and clinically significant increase in body temperature.

Descriptive statistics were used to summarize dose of *B. anthracis* spores, survival time, time to onset of bacteremia (by culture and by PCR), time to detectable serum PA, and time to clinically significant increase in body temperature. Possible relationships between clinical observations (survival time, time to 1st detected bacteremia by culture and by PCR, time to 1st significant temperature increase, and serum PA kinetic parameters) were evaluated using Spearman's correlation coefficients.

Results

The 8 monkeys were challenged with a range of 167 x LD_{50} to 451 x LD_{50} dose of *B. anthracis* (Ames strain) spores. The average exposure for all challenged monkeys was 260 ± 108 x LD_{50}.

Six of 8 monkeys died within the 30-day study interval; 2 monkeys survived to the end of the study interval. The earliest time of death was 85 hours and the longest time until death while on study was 156 hours. The survivors were alive at the last time point on study, 720 hours. Notably, the 2 animals that survived until the end of the study did not have the lowest spore challenge LD_{50} values. Moreover, for the monkeys that died, survival time was not significantly correlated with challenge dose ($p > 0.8401$).

Gross necropsy was performed on all monkeys that died. In addition, histopathology was performed upon 2 monkeys that did not have gross lesions typical of anthrax, even though both monkeys were bacteremic several days prior to death. Both monkeys had several microscopic lesions typical of anthrax, including presence of large bacilli, edema, fibrin, hemorrhage(s), and suppurative inflammation in various organs. Lymphoid necrosis was present in lymph nodes and spleen; these findings were also consistent with anthrax infection. All other monkeys had gross lesions typical of inhalation anthrax.

All monkeys became bacteremic by culture and by PCR during the study; 1 monkey had less than 5 colonies at 2 timepoints by culture and no other samples were positive. Bacteremia

by culture or PCR was observed as early as 30 hours post challenge and the median time to bacteremia was 36 and 39 hours, respectively. In the majority of animals, the onset of positive bacteremia by PCR or culture and 1st detectable serum PA occurred concurrently (Figure 13-13).

All 8 monkeys had serum PA detected after spore challenge: 3 of the 8 monkeys had detectable PA levels at 30 hours post challenge and all animals had detectable PA levels by 48 hours post challenge (Figure 13-13). The median time to 1st detection of serum PA was 39 hours. In animals that died, and had terminal blood samples, the serum PA concentrations were in excess of 8,000 ng/mL. In the 2 animals that survived, PA was 1st detected at 42 and 48 hours post challenge, respectively. PA concentrations in these animals (peak 102-139 ng/mL) were not as high as in animals that died (highest measured concentrations prior to death of 211-1162 ng/mL). Serum PA values returned to below the limit of quantitation in the 2 survivors by Days 14 and 21, coincident with or following attainment of measurable neutralizing anti-PA responses in these animals.

Of the hematology parameters, WBC was the most indicative of disease progression. WBC was relatively stable in all of the animals through 24 hours post spore challenge. From 24-36 hours, most animals exhibited sharp declines in WBC. These decreases were followed over the next 2 days by increases in WBC to levels at or exceeding baseline values. In the 2 animals that survived, WBC normalized as the animals recovered (Figure 13-13).

Elevations in CRP were observed in most of the animals that died while on study and the increases in CRP generally trailed the onset of bacteremia and temperature increase (Figure 13-13).

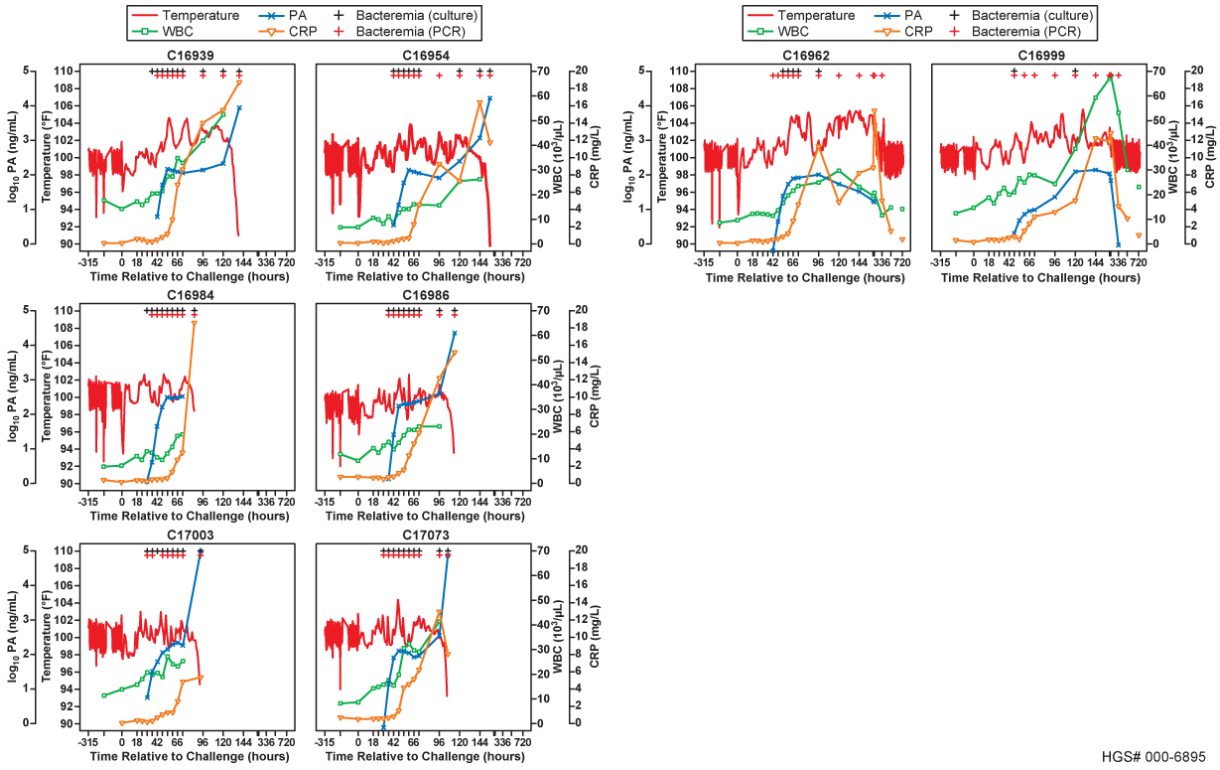

Figure 13-13 Time course of clinical parameters in monkey model of inhalation anthrax (685-G005762)

Illustration of key clinical parameters in individual monkeys following anthrax spore challenge. Left panels, monkeys that died on study; right panels, monkeys that survived the study period.

The mean serum PA concentration-time profile for the animals who died shows an initial rise up to about 48 hours post challenge, followed by a period during which the concentrations plateau, which in turn is followed by a 2nd period of rising concentrations. Examination of the individual concentration-time profiles shows that a similar pattern of rise-plateau-rise can be observed in the profiles for 4 of the 6 monkeys that died. To examine a possible relationship between PA kinetics and magnitude of the spore challenge administered to the monkeys, correlation analysis was performed. For the animals that died, there were no significant correlations between serum PA kinetic parameters and the magnitude of the spore challenge.

Toxin neutralization activity, a measure of the monkey's immune response to PA, was measured in samples drawn pre-challenge and at Days 14, 21, and 30 for animals that survived to Day 14 or greater. All animals had values below the limit of detection prior to challenge. Only 2 animals were alive at the Day 14 to have samples taken and TNA was measurable in both animals. At Days 21 and 30, TNA titers were > 5000, indicating that both of the surviving animals had mounted a strong immune response to PA (Table 13-15).

Table 13-15 Toxin neutralization assay (Study 685-G005762)

		TNA (Titer)		
	Pre-Challenge	Day 14	Day 21	Day 30
C16962	BLOD	4624	> 5000	> 5000
C16999	BLOD	> 5000	> 5000	> 5000

BLOD = below the limit of detection.

Survival time was strongly correlated with the time to detection of PA ($r = 0.93$, $p = 0.0008$) indicating that the appearance of serum PA was a good predictor of ensuing death. Detection of bacteremia measured by culture was consistent with bacteremia detection by PCR and survival time was strongly correlated with time to bacteremia by culture ($r = 0.96$, $p = 0.0001$) and by PCR ($r = 0.85$, $p = 0.0082$). Survival time was not correlated with time to a significant increase in body temperature ($r = -0.30$, $p = 0.4685$ for 1st significant temperature rise for consecutive temperature elevations) likely due to the diurnal temperature rhythms in monkeys.

Bacteremia by culture or by PCR and serum PA appear to be the 1st indicators of disease, followed shortly thereafter by changes in WBC and subsequently by increases in CRP (Figure 13-14). The sequence of these events was similar in all animals (Figure 13-13). For the 2 surviving animals, after these initial changes, all of the clinical parameters (serum PA, bacteremia by culture and PCR, temperature, WBC, and CRP) returned to normal as the animals recovered. Of note, bacterial DNA as measured by PCR persisted at measurable levels longer than either bacteria measured by culture or toxemia by serum PA.

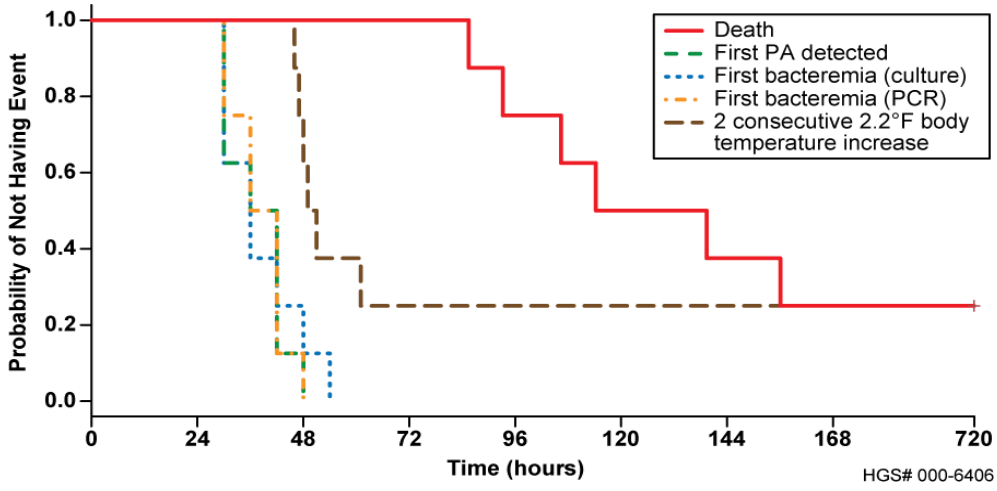

Figure 13-14 Time to event for clinical parameters in anthrax-challenged monkeys (685-G005762)

13.10 Appendix 10: Pivotal Animal Efficacy Studies

13.10.1 Efficacy of Therapeutic Treatment with Raxibacumab in the Rabbit (Study 682-G005758)

This GLP study, "Evaluation of Raxibacumab Efficacy as Therapeutic Treatment against Inhalation Anthrax in the Rabbit Model," evaluated the efficacy of raxibacumab when administered as a therapeutic treatment against lethality due to inhalation exposure to *B. anthracis* in rabbits.

Study Design and Analysis

This was an open-label, parallel-group, randomized, placebo-controlled GLP study to evaluate the therapeutic efficacy of a single IV dose of raxibacumab in anthrax spore challenged rabbits experiencing symptoms of inhalation anthrax. Fifty-four NZW rabbits were to be randomized by gender and body weight into each of 3 treatment groups (18 rabbits/group, 50% males and 50% females) and challenged with a targeted 200 x LD_{50} dose of *B. anthracis* spores (Ames strain). The trigger for treatment of individual rabbits with raxibacumab or placebo was detectable serum PA or 1st rise in body temperature of 2°F or more above the baseline average at 2 consecutive timepoints (whichever occurred first). For each rabbit, a single IV treatment of 20 or 40 mg/kg raxibacumab or placebo was administered immediately following detection of serum PA or body temperature increase.

The raxibacumab administered as a single IV dose was produced by the same manufacturing process as that proposed for licensure. The placebo was raxibacumab formulation buffer, also administered as a single IV dose. Placebo was administered in a volume equal in mL/kg volume as the 40 mg/kg raxibacumab dose.

The primary efficacy variable was survival at Day 14, defined as the percent of rabbits alive at Day 14. The secondary efficacy endpoint was survival time, defined as the time from spore challenge to death during the 14-day period. For analysis of the primary efficacy endpoint, survival at Day 14 was compared between the placebo group and each of the raxibacumab treatment groups in the ITT population defined as all monkeys that were randomized and spore challenged, using a 2-sided Fisher's exact test and was subject to multiple comparison adjustment using the Hochberg procedure. Under the Hochberg procedure, the results were to be considered statistically significant if at least 1 of the pairwise comparisons between raxibacumab and placebo achieved a p-value < 0.025, or both pairwise comparisons between raxibacumab and placebo achieved a p-value < 0.05. For the secondary endpoint analysis, the log-rank test was used to compare survival time over 14 days between the placebo group and each of the active treatment groups and was not subject to any multiple comparison procedures.

Results

Fifty-four rabbits were enrolled in the study. One rabbit died due to a blood clot resulting from implantation of the venous access port. The remaining 53 rabbits were randomized into 3 groups (placebo (n = 17), 20 mg/kg raxibacumab (n = 18), and 40 mg/kg raxibacumab

(n = 18). The ratio of males:females and mean values for weight were comparable among treatment groups and all of the rabbits were of the same age. The rabbits were challenged with a mean of 228.1 ± 41.5 x LD_{50} of anthrax spores (the range of mean spore doses across groups was 221.5 to 233.0 x LD_{50}).

The groups were not statistically different with regard to use of trigger event or time to treatment initiation nor were they different with regard to signs and symptoms at or before treatment initiation. The majority of rabbits in each group was bacteremic and/or toxemic, and had elevated body temperature at or before treatment initiation. There were no rabbits with temperature increase that were not also bacteremic or toxemic at or before treatment. All rabbits developed trigger events and were treated within 36 hours post spore challenge.

The primary efficacy endpoint in this study was survival at Day 14 in the ITT population. The 14-day survival rates were 0%, 27.8%, and 44.4% in the placebo, 20 mg/kg raxibacumab, and 40 mg/kg raxibacumab groups, respectively (Table 13-16 and Figure 13-15). The primary endpoint was met: the survival rates at Day 14 were statistically significantly higher in the 40 mg/kg raxibacumab group (p = 0.0029) and the 20 mg/kg raxibacumab group (p = 0.0455) compared with the placebo group.

There was a statistically significant increasing trend in survival at Day 14 across the placebo, 20 mg/kg raxibacumab, and 40 mg/kg raxibacumab groups (p = 0.0027 from Cochran-Armitage trend test). Although the relative survival rate in the 40 mg/kg raxibacumab group was 160% compared with the 20 mg/kg raxibacumab group, this difference was not statistically significant (p = 0.2962, from the likelihood ratio test).

Table 13-16 Survival at Day 14 (682-G005758)

Treatment	N	Number (%) of Survivors	P-Value[1]
Placebo	17	0 (0.0%)	-
20 mg/kg raxibacumab	18	5 (27.8%)	0.0455
40 mg/kg raxibacumab	18	8 (44.4%)	0.0029

[1] Based on 2-sided Fisher's exact test for the comparison vs the control group. P-value = 0.2962 from likelihood ratio test for comparison of survival at Day 14 between the 20 mg/kg and 40 mg/kg raxibacumab treatment groups.

P = 0.0027 based on Cochran-Armitage trend test across the 3 groups.

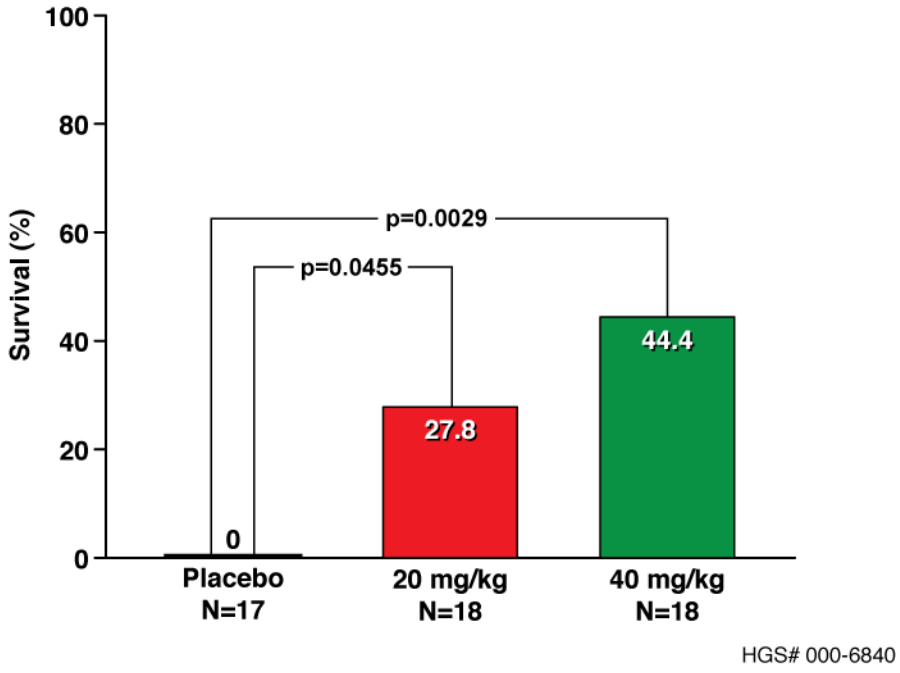

Figure 13-15 Survival at Day 14 (682-G005758)

Because 1 rabbit that was randomized to the placebo group inadvertently received 40 mg/kg raxibacumab, an as-treated analysis was performed including this animal in the 40 mg/kg raxibacumab group. The as-treated analysis also excluded 1 animal in the 20 mg/kg raxibacumab group that died of a broken back during spore challenge before receiving any study agent. The 14-day survival rates were 0%, 29.4%, and 42.1% in the placebo, 20 mg/kg raxibacumab, and 40 mg/kg raxibacumab groups, respectively. In the as-treated analysis the survival rate at Day 14 again was significantly higher in both the 40 mg/kg raxibacumab group ($p = 0.0038$) and the 20 mg/kg raxibacumab group ($p = 0.0445$) compared with the placebo group.

The results of the as-randomized analysis (including the rabbit that died prior to spore challenge) were also consistent with those observed in the ITT analysis. The 14-day survival rate was 0%, 27.8%, and 44.4% in the placebo, 20 mg/kg raxibacumab, and 40 mg/kg raxibacumab groups, respectively. Survival at Day 14 was significantly higher in both the 40 mg/kg raxibacumab group ($p = 0.0029$) and the 20 mg/kg raxibacumab group ($p = 0.0455$) compared with the placebo group.

Several subgroups by bacteremia and toxemia status at the time of treatment were specified. Figure 13-16 displays the absolute improvement in survival at Day 14 (point estimate and 95% confidence intervals [CI]) of the 20 and 40 mg/kg treatment groups compared with placebo for the prespecified subgroups of the ITT population. The primary efficacy analysis in the ITT population is displayed at the top of the figure and the results in the prespecified subgroups are displayed below it. The dashed vertical line represents the point estimate for the

treatment effect in the ITT population. The solid vertical line indicates 0% added benefit. Consequently, point estimates to the right of the solid vertical line (shown as X) indicate an improved survival benefit compared with placebo. Horizontal lines that cross the vertical line convey that the 95% confidence interval includes 0% benefit. Horizontal lines that cross the dashed vertical line convey that the effect in these subgroups is included within the effect in the overall population.

In Figure 13-16, for all of the prespecifed subgroups by toxemia, bacteremia, or increased temperature status at the time of study agent administration, the point estimate of the survival benefit is contained within the confidence intervals of the main effect. This demonstrates that the survival benefit in the prespecified subgroups is consistent with the effect observed in the overall ITT population. Also in all subgroups, the 40 mg/kg treatment group had a higher point estimate for survival than the 20 mg/kg treatment group, although the confidence intervals for the 2 dose groups overlap, indicating that the differences between the raxibacumab treatment group point estimates were not statistically significant.

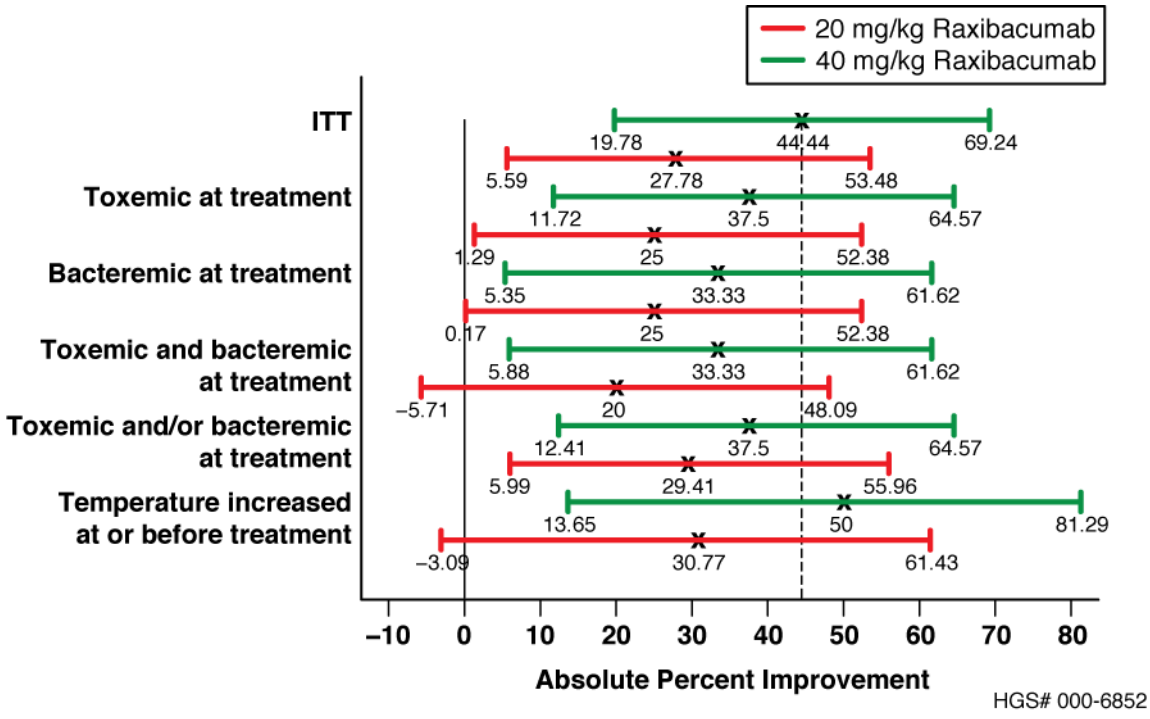

Figure 13-16 Absolute improvement in Day 14 survival by subgroup (prespecified) and unconditional exact 95% CI compared with placebo (682-G005758)

Solid vertical line: 0% benefit.

Dashed vertical line: point estimate of the survival benefit in the overall (ITT) population.

The 95% confidence intervals (CI) were calculated using an unconditional exact method. For 95% CIs that excluded 0, the statistical significance should be interpreted according to results from the Fisher's exact test based on a conditional method.

The secondary efficacy endpoint was survival time, defined as the time from spore challenge to death during the 14-day period. In the ITT analysis (Figure 13-17), the survival time was significantly longer in the 20 mg/kg group (median = 3.5 days, p = 0.0181) and the 40 mg/kg group (median = 3.8 days, p = 0.0034) compared with the placebo group (median = 2.7 days). In addition, survival was significantly higher for the overall raxibacumab treatment effect compared with placebo (p = 0.0064).

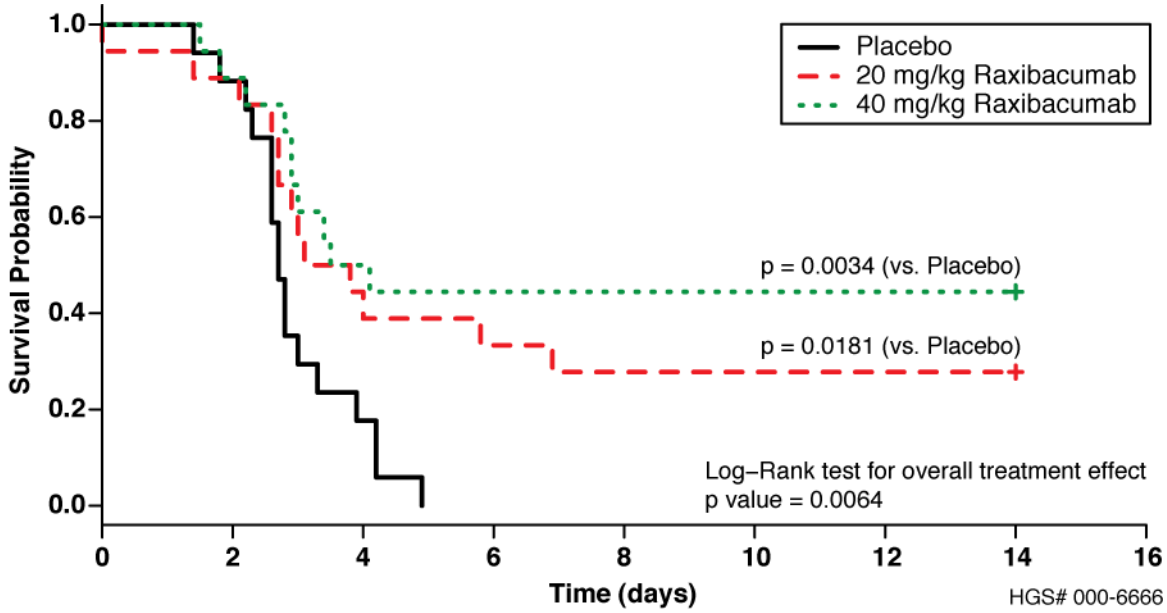

Figure 13-17 Survival curves (682-G005758)

With respect to bacteremia, of the 52 treated rabbits, 46 were positive for bacteremia by blood culture at or before the time of treatment. The majority of survivors (all raxibacumab-treated) had negative *B. anthracis* blood cultures by 10 to 24 hours post treatment, and all had negative blood cultures at the end of the study.

PA levels over time by individual rabbit are provided for placebo-treated rabbits (all non-survivors), raxibacumab-treated non-survivors, and raxibacumab-treated survivors in Figure 13-18. PA values rose sharply from 10-20 hours post challenge. Among placebo-treated rabbits, PA values exhibited a slight plateau during the period from 20 to 50 hours post challenge. This was followed by a 2nd rapid increase until death. Among the raxibacumab-treated non-survivors, the plateau period lasted longer (approximately 20 to 80 hours post challenge), and was generally not followed by the 2nd rise. Among raxibacumab-treated survivors, the plateau period (whose duration was the same as that for raxibacumab-treated non-survivors) was followed by a rapid decline for the majority of rabbits until the end of the study.

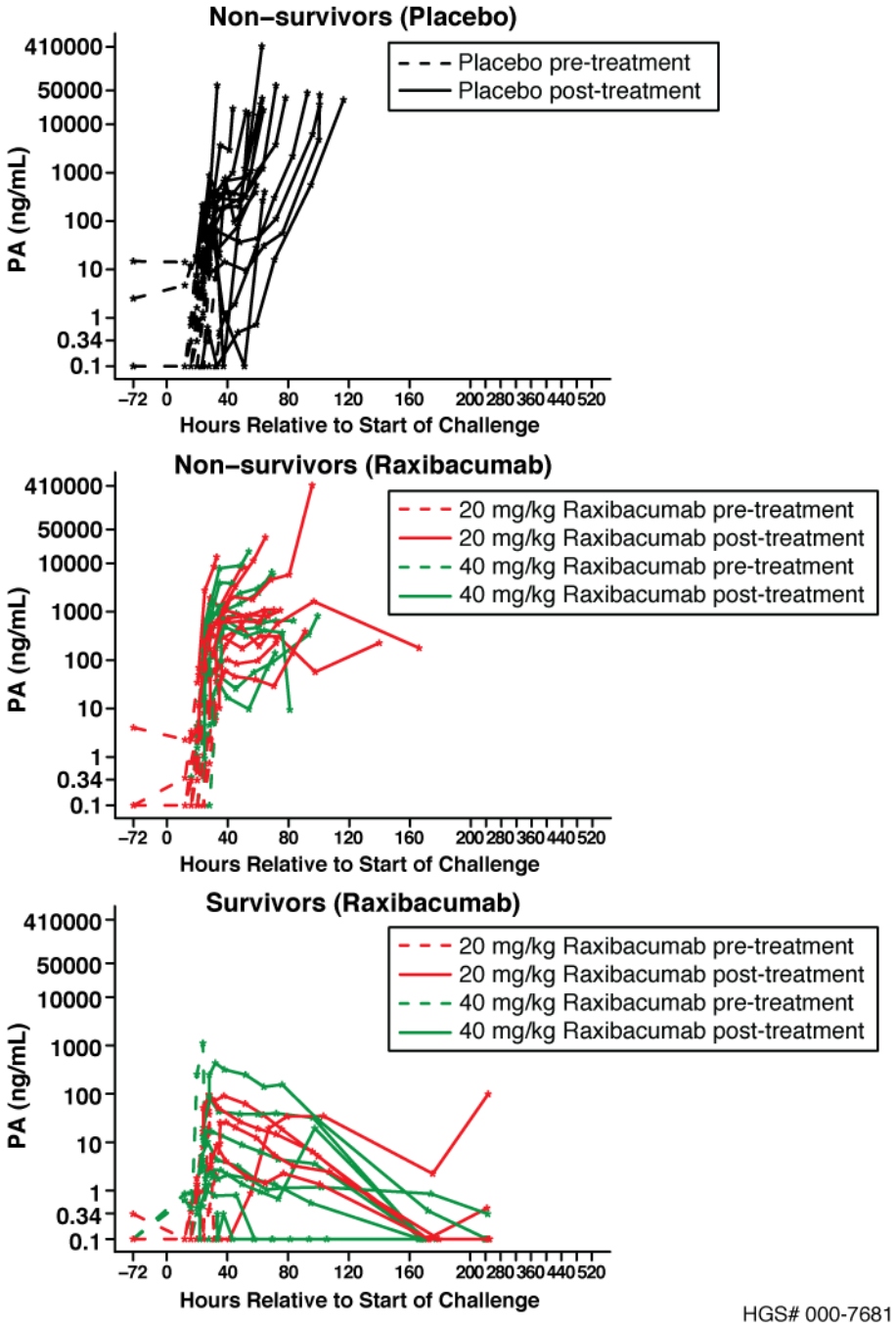

Figure 13-18 PA levels over time by individual rabbit (682-G005758)

Clear signs of inflammation and activation of the immune system were seen in all rabbits. Evidence of this is seen in the initial drop followed by a sharp rise in WBCs and elevation of

CRP in all rabbits. Among the survivors, these parameters returned or otherwise trended toward returning to baseline near the end of the study. The most common adverse clinical observations were inappetence, lethargy, and rapid respirations.

All of the animals that died or were euthanized moribund had findings consistent with anthrax disease by gross necropsy or microscopic examination and the gross and histopathology was qualitatively the same as reported for rabbits succumbing to inhalation anthrax (Zaucha et al, 1998). Microscopic examination was performed both by BBRC as part of the protocol for Study 682-G005758 and separately by EPL (Study 866-001) in response to a request by FDA for an independent and blinded review of the histopathology. In both readings, the incidence and severity of histopathology findings was the same or greater in the placebo treatment group for all tissues, except brain, in which the incidence and/or severity of bacteria, hemorrhage and inflammation was greater in the raxibacumab-treated groups. The incidence and severity of brain findings was not greater in the 40 mg/kg raxibacumab treatment group than in the 20 mg/kg raxibacumab treatment group. Raxibacumab did not cause more rapid death among non-survivors and the median time to death was numerically greater in the raxibacumab treatment groups compared with the placebo group: 2.9 days, 40 mg/kg raxibacumab; 3.0 days, 20 mg/kg raxibacumab; 2.7 days, placebo. Importantly, in the surviving animals, all of which were treated with raxibacumab (there were no survivors in the placebo treatment group) clinical and laboratory parameters (eg, temperature, hematology, CRP, serum PA) had normalized or were returning to normal values at the end of the study and there were no remarkable findings (eg, seizures, loss of balance, loss of coordination) related to central nervous system effects in the surviving rabbits while on study or at the end of the study period.

With respect to MICs for levofloxacin and ciprofloxacin, for levofloxacin, the MICs prior to treatment and the terminal MICs ranged from 0.50 to 4.00 µg/mL. For ciprofloxacin, the MICs prior to treatment ranged from 0.25 to 2.00 µg/mL, and the terminal MICs ranged from 0.125 to 2.00 µg/mL, demonstrating that the sensitivity of the bacteria to these antibiotics did not change during the course of the study.

Conclusions

Efficacy

- The primary endpoint of survival at Day 14 was met, with a statistically significant proportion of rabbits surviving in the 20 mg/kg raxibacumab group (28%, p = 0.0455) and 40 mg/kg raxibacumab group (44%, p = 0.0029) compared with the placebo group (0%).
- There was an increasing trend in survival at Day 14 across the placebo, 20 mg/kg raxibacumab, and 40 mg/kg raxibacumab groups (p = 0.0027). However, the difference in the survival at Study Day 14 between the 2 raxibacumab groups was not statistically significant (p = 0.2962).
- The raxibacumab survival benefit was robust and observed across all prespecified subgroups, including:
  - rabbits that were toxemic at treatment initiation.

  - rabbits that were bacteremic confirmed by culture at treatment initiation.
  - rabbits that were toxemic and bacteremic at treatment initiation.
  - rabbits that were toxemic and/or bacteremic at treatment initiation.
- In addition, survival benefit was observed for the subgroup of rabbits that had temperature increases at or before treatment initiation.
- Survival time was significantly prolonged in the 20 mg/kg raxibacumab group (median = 3.5 days, p = 0.0181) and 40 mg/kg group (median = 3.8 days, p = 0.0034) compared with the placebo group (median = 2.7 days).

Other Observations

- The majority of survivors (all raxibacumab-treated) had negative *B. anthracis* blood cultures by 10 to 24 hours post treatment, and all had negative blood cultures at the end of the study.
- Clear signs of inflammation and activation of the immune system were seen in all rabbits. Among the survivors, these parameters returned or trended to baseline by the end of the study.
- Gross findings at necropsy were consistent with death due to inhalation anthrax and histopathology revealed microscopic lesions typical of inhalation anthrax.

13.10.2 Efficacy of Therapeutic Treatment with Raxibacumab in the Monkey (Study 724-G005829)

This GLP study, "Evaluation of Raxibacumab Efficacy as Therapeutic Treatment against Inhalation Anthrax in the Cynomolgus Macaque" evaluated the efficacy of raxibacumab when administered as a therapeutic treatment against lethality upon appearance of clinical symptoms due to inhalation exposure to *B. anthracis* in cynomolgus monkeys.

Study Design and Analysis

This was a blinded, parallel-group, randomized, placebo-controlled GLP study to evaluate the therapeutic efficacy of a single IV raxibacumab dose in anthrax spore inhalation-challenged monkeys experiencing symptoms of inhalation anthrax. Forty naïve cynomolgus monkeys were to be randomized into 2 separate groups of 14 monkeys and 1 group of 12 monkeys (50% male, 50% female in each group) and challenged with a targeted 200 x LD_{50} dose of *B. anthracis* spores (Ames strain). For individual monkeys, upon detection of serum PA (therapeutic trigger symptom), a single 1 mg/kg IM dose of diphenhydramine was to be administered, followed within 5 minutes by a single bolus IV injection of either 40 mg/kg or 20 mg/kg raxibacumab, or placebo. The Sponsor, the Battelle Study Director, and all staff administering study agent to and/or conducting assessments of study monkeys post challenge were to be blinded to the animal treatment assignments.

The raxibacumab administered as a single IV dose was produced by the same manufacturing process as that proposed for licensure. The placebo was raxibacumab formulation buffer, also administered as a single IV dose. Placebo was administered in a volume equal in mL/kg volume to the 40 mg/kg raxibacumab dose.

The primary efficacy variable was survival at Day 28 defined as the percent of monkeys alive at Day 28. The secondary efficacy endpoint was survival time, defined as the time from spore challenge to death during the 28-day period. The primary analysis was performed on the ITT population, defined as all monkeys that were randomized and challenged with anthrax spores. All deaths observed after the monkeys were challenged with anthrax spores, regardless of administration of study agent, were defined as treatment failures in the primary analysis. The primary efficacy analysis was performed using the Fisher's exact test, and was subject to multiple comparison adjustment using the step-down sequential testing procedure. The 40 mg/kg raxibacumab group was compared with the placebo group (2-sided $\alpha = 0.05$). If the result was statistically significant, superiority of 40 mg/kg vs placebo was established. The 20 mg/kg raxibacumab group was then compared with the placebo group (2-sided $\alpha = 0.05$). If the result was statistically significant, superiority of 20 mg/kg raxibacumab vs placebo was established. For the secondary efficacy endpoint analysis, the log-rank test was used to compare survival time over 28 days between the placebo group and each of the active treatment groups. For animals that were alive at the end of study, survival time was censored at Study Day 28. The analysis of the secondary efficacy endpoint was performed at a significance level of 0.05 and was not subject to any multiple comparison procedure.

Results

In total, 40 monkeys were screened, randomized, and challenged with anthrax spores in the study (12 in the placebo group, and 14 in each of the 20 and 40 mg/kg raxibacumab groups). All monkeys received treatment according to their assigned group. The ratio of males:females and mean values for age and weight were comparable among treatment groups. The monkeys were challenged with a mean of 184.0 ± 46.8 x LD_{50} anthrax spores.

Across the 3 treatment groups there were no statistically significant differences in time to treatment, events triggering treatment initiation, or the onset of bacteremia and toxemia. Thirty-seven of 40 animals on this study exhibited positive serum PA levels by 54 hours post-median challenge time. The remaining 3 monkeys all tested positive for bacteremia by culture while on study. The groups were also similar with respect to signs and symptoms around the time of treatment.

The primary efficacy endpoint in this study was survival at Day 28 in the ITT population. The primary endpoint was met: survival at Day 28 was higher in both the 40 mg/kg raxibacumab group (64.3%, $p = 0.0007$) and the 20 mg/kg raxibacumab group (50.0%, $p = 0.0064$) compared with the placebo group (0%), as shown in Table 13-17 and Figure 13-19.

There was a statistically significant increasing trend in survival at Day 28 across the placebo, the 20 mg/kg raxibacumab group, and 40 mg/kg raxibacumab group ($p = 0.0011$ from Cochran-Armitage trend test). Although the relative survival rate in the 40 mg/kg raxibacumab group was 129% compared with the 20 mg/kg raxibacumab group, this difference was not statistically significant ($p = 0.4441$ from the likelihood ratio test).

Table 13-17 Survival at Day 28 (724-G005829)

Treatment	N	Number(%) of Survivors	P-value[1]
Control	12	0 (0.0%)	-
20 mg/kg raxibacumab	14	7 (50.0%)	0.0064
40 mg/kg raxibacumab	14	9 (64.3%)	0.0007

[1] P-value based on 2-sided Fisher's exact test for the comparison vs the placebo group.

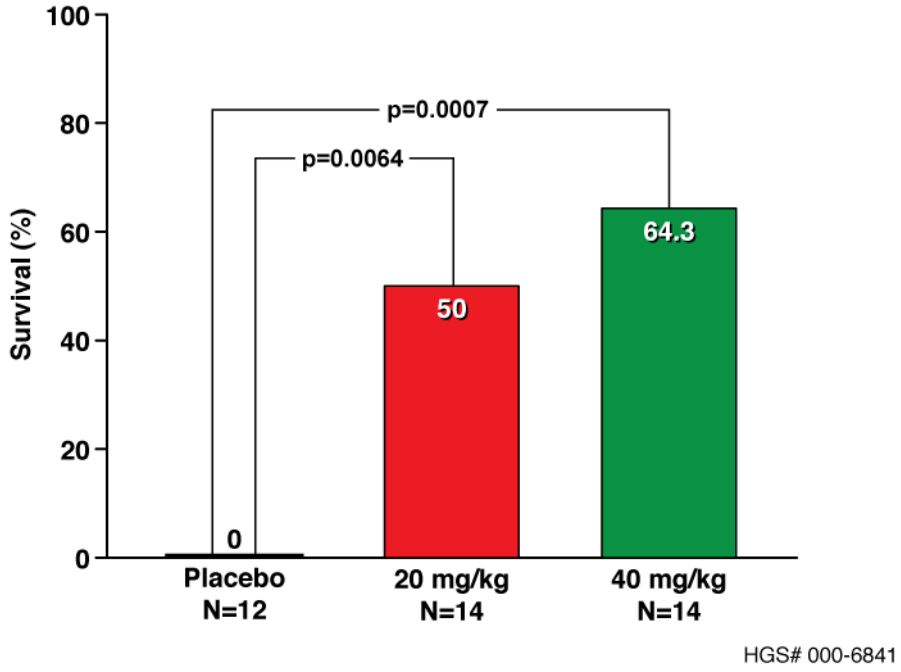

Figure 13-19 Survival at Day 28 (724-G005829)

All randomized monkeys in the study were challenged with *B. anthracis* spores and received study agent according to their planned treatment group, therefore the as-treated population is identical to the ITT population.

The mean inhaled dose of *B. anthracis* spores in the 40 mg/kg raxibacumab group (157 x LD_{50}) was lower than the 198 x LD_{50} mean dose in the placebo group and 199 x LD_{50} mean dose in the 20 mg/kg raxibacumab group, a difference that reached statistical significance ($p = 0.0255$). This finding prompted a sensitivity analysis of the primary endpoint in the anthrax dose-adjusted population. The results of the anthrax dose-adjusted analysis, which excluded 3 monkeys with a spore dose below the lowest dose received in the placebo group, were consistent with those observed in the ITT analysis, with survival at Day 28 significantly higher in both the 40 mg/kg raxibacumab group (72.7%, $p = 0.0003$) and the 20 mg/kg raxibacumab group (50%, $p = 0.0064$) compared with the placebo group (0%). The Cox model which included all 40 monkeys showed that, adjusting for the dose of *B. anthracis*,

the survival benefit remained statistically significant in the 40 mg/kg raxibacumab group ($p = 0.0018$) and the 20 mg/kg raxibacumab group ($p = 0.0036$) compared with the placebo group.

Several subgroups by bacteremia and toxemia status at the time of treatment were specified. Figure 13-20 displays the absolute improvement in survival at Day 26 (point estimate and 95% confidence intervals) of the 40 mg/kg treatment group and the 20 mg/kg treatment group compared with placebo for the prespecified subgroups of the ITT population. For all of the prespecifed subgroups by toxemia, bacteremia, or increased temperature status at the time of study agent administration, the point estimate of the survival benefit is contained within the confidence intervals of the main effect. This demonstrates that the survival benefit in the prespecified subgroups is consistent with the effect observed in the overall ITT population. Also in all subgroups, the 40 mg/kg treatment group had a higher point estimate for survival than the 20 mg/kg treatment group, although the confidence intervals for the 2 dose groups overlap, indicating that the differences between the raxibacumab treatment group point estimates were not statistically significant.

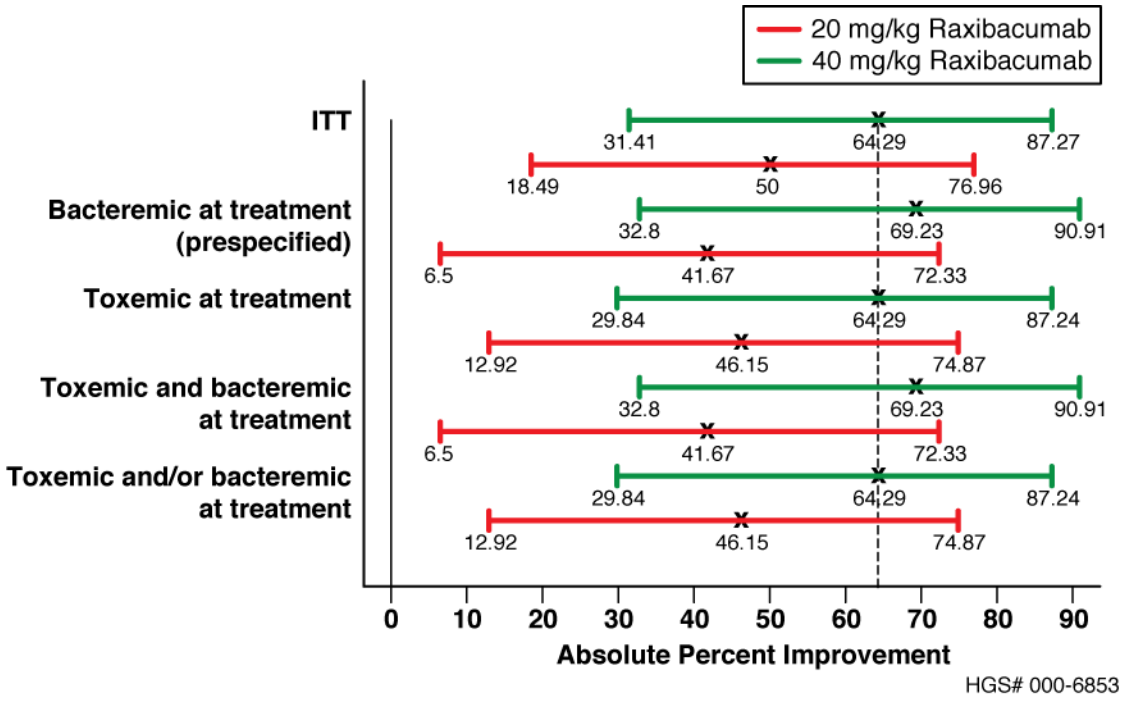

Figure 13-20 Absolute improvement in Day 28 survival by subgroup (prespecified) and unconditional exact 95% CI compared with placebo (724-G005829)

Solid vertical line: 0% benefit.

Dashed vertical line: point estimate of the survival benefit in the overall (ITT) population.

The 95% confidence intervals (CI) were calculated using an unconditional exact method. For 95% CIs that excluded 0, the statistical significance should be interpreted according to results from the Fisher's exact test based on a conditional method.

The secondary efficacy endpoint was survival time defined as the time from the beginning of spore challenge to death during the 28-day study period. As shown in Figure 13-21, survival time was significantly longer in the 20 mg/kg raxibacumab ($p = 0.0029$) and 40 mg/kg ($p = 0.0004$) groups compared with the placebo group. The median survival time was 3.3 days in the placebo group. The median survival times in the raxibacumab groups could not be quantified because at least half of the monkeys remained alive at Day 28 in the 20 and 40 mg/kg raxibacumab groups, 50% and 64%, respectively. This means that the median survival times extended beyond 28 days in the 2 raxibacumab groups. Per protocol, survivors were to be observed for an additional 60 days in a BL-2 setting. There were no additional deaths in the 60-day observation period.

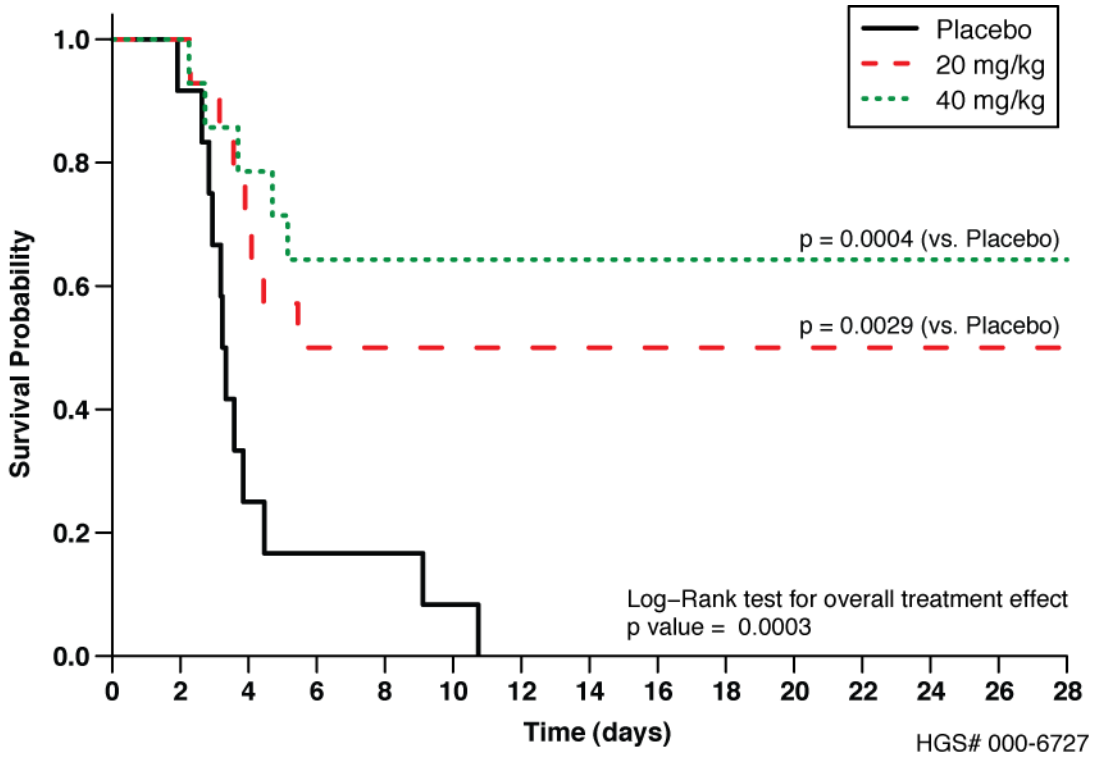

Figure 13-21 Survival curves (724-G005829)

With respect to bacteremia, the incidence of positive blood cultures was 100% across all dose groups at some point post challenge and 35 of 40 monkeys (88%) had anthrax bacteremia prior to treatment; 13 of 14 monkeys in the 40 mg/kg raxibacumab group (93%), 12 of 14 monkeys in the 20 mg/kg raxibacumab group (86%), and 10 of 12 monkeys in the placebo group (83%) were positive for blood cultures prior to treatment.

All monkeys found dead or euthanized had a terminal bacteremia by blood culture. Bacterial cultures were positive for 3 tissues (liver, spleen and lymph nodes) assessed in 23 of the 24 animals that died.

PA levels over time by individual monkey are provided for placebo-treated monkeys (all non-survivors), raxibacumab-treated non-survivors, and raxibacumab-treated survivors in Figure 13-22. There were no measurable serum PA concentrations in specimens collected 3 days prior to the spore challenge for any monkey. PA values spiked sharply just prior to 40 hours post challenge. Among some placebo-and raxibacumab-treated non-survivors, PA values exhibited a brief plateau during the period from 40 to 80 hours post challenge. For non-survivors the plateau period was generally followed by a 2nd spike in PA levels that lasted until death. Among raxibacumab-treated non-survivors the 2nd spike was more gradual, though it nonetheless continued to rise until death. Among raxibacumab-treated survivors, the 2nd spike in PA did not occur; rather, the PA declined to undetectable levels for the majority of monkeys by the end of the study.

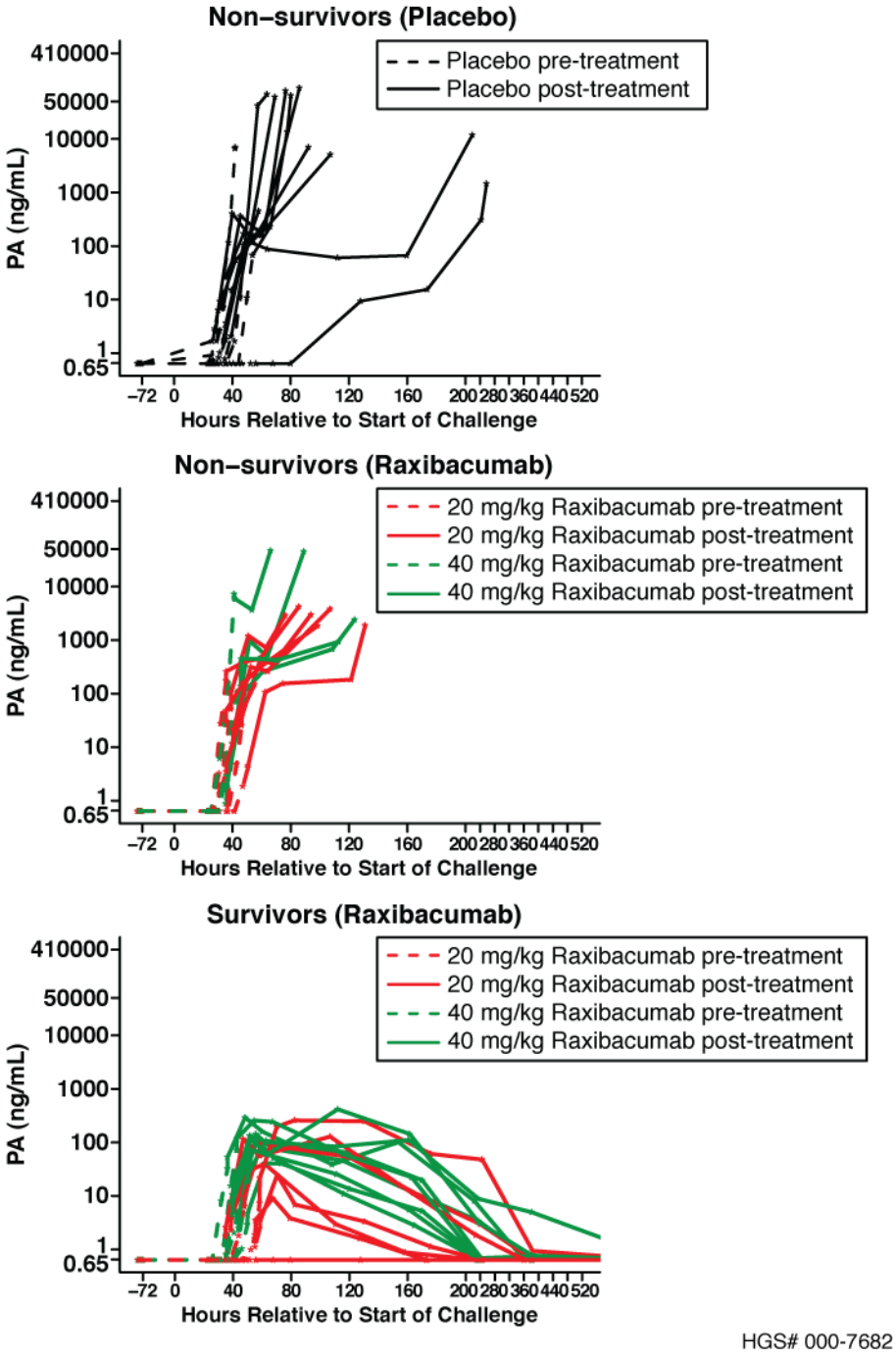

Figure 13-22 PA levels over time by individual monkey (724-G005829)

Monkeys in this study generally experienced outward clinical signs consistent with inhalation anthrax, and those that succumbed to disease followed the hallmark progression pattern characteristic of inhalation anthrax.

All of the animals that died or were euthanized moribund had findings consistent with anthrax disease by gross necropsy or microscopic examination and the gross and histopathology was qualitatively the same as reported for non-human primates succumbing to inhalation anthrax (Friedlander, 1993; Fritz et al, 1995; Twenhafel et al, 2007; Vasconcelos et al, 2003). Microscopic examination was performed initially by Battelle only on non-surviving animals that did not have evidence of anthrax disease on gross necropsy, which comprised 2 animals. At the request of FDA, histopathology was examined on all non-surviving animals. This was performed as an independent blinded review of the slides by EPL as Study 866-002. In both readings, the incidence and severity of histopathology findings was the same or greater in the placebo treatment group for all tissues, except brain in which the severity of bacteria, hemorrhage and inflammation was higher in the raxibacumab-treated animals, as assessed by BBRC and independently by EPL. The severity of brain findings was not greater in the 40 mg/kg raxibacumab treatment group than in the 20 mg/kg raxibacumab treatment group. Raxibacumab did not cause more rapid death among non-survivors and the median time to death was numerically greater in the raxibacumab treatment groups compared with the placebo group: 3.7 days, 40 mg/kg raxibacumab; 3.9 days, 20 mg/kg raxibacumab; 3.3 days, placebo. Importantly, in the surviving animals, all of which were treated with raxibacumab (there were no survivors in the placebo treatment group) clinical and laboratory parameters (eg, temperature, hematology, CRP, serum PA) had normalized or were returning to normal values at the end of the study and there were no remarkable findings (eg, seizures, loss of balance, loss of coordination) related to central nervous system effects in the surviving monkeys while on study or at the end of the study period.

Serum toxin neutralization assay (TNA) titers were measured in surviving animals to determine if the monkeys had mounted an immune response to PA. Results for the 20 and 40 mg/kg raxibacumab groups are provided in Table 13-18. The mean serum TNA titer for the 40 mg/kg dose group was nearly 3-fold higher than that for the 20 mg/kg dose group, but this difference did not attain statistical significance, as shown by the overlap in 95% CI, likely due to the small number of animals included in the comparison. As shown by the increase in mean serum TNA titer for the 40 mg/kg dose group relative to the 20 mg/kg raxibacumab group, increasing exposure to raxibacumab did not prevent the animals from mounting a TNA response.

Table 13-18 Summary of serum TNA titers in surviving monkeys (724-G005829)

		Serum TNA Titer	
		Predose	28 Day Postdose
20 mg/kg	n	7	7
	Mean	0	5328
	95% CI	(0, 0)	(523, 10133)
40 mg/kg	n	9	9
	Mean	0	14827
	95% CI	(0, 0)	(5163, 24490)

The MIC results confirm that bacteria cultured from the blood of aerosol-challenged animals was inhibited by similar concentrations of levofloxacin and ciprofloxacin as the original challenge material. For levofloxacin, MICs prior to treatment and terminal MICs ranged from 0.50 to 4.00 μg/mL. For ciprofloxacin, the MICs prior to treatment and terminal MICs ranged from 0.25 to 2.00 μg/mL.

Conclusions

Efficacy

- The primary endpoint of survival in the ITT population at Day 28 was met, with a statistically significant proportion of monkeys surviving in both the 40 mg/kg raxibacumab group (64.3%, p = 0.0007) and the 20 mg/kg raxibacumab group (50.0%, p = 0.0064) compared with the placebo group (0%).
- There was an increasing trend for survival at Day 28 across the placebo, the 20 mg/kg raxibacumab, and the 40 mg/kg raxibacumab groups (p = 0.0011). However, the difference in the survival at Study Day 28 between the 2 raxibacumab groups was not statistically significant (p = 0.4441).
- The results of the anthrax dose-adjusted analysis, which excluded 3 monkeys with a spore dose below the lowest dose received in the placebo group, were consistent with those observed in the ITT analysis, with survival at Day 28 significantly higher in both the 40 mg/kg raxibacumab group (72.7%, p = 0.0003) and the 20 mg/kg raxibacumab group (50%, p = 0.0064) compared with the placebo group (0%). The Cox model which included all 40 monkeys showed that, adjusting for the dose of *B. anthracis*, the survival benefit remained statistically significant in the 40 mg/kg raxibacumab group (p = 0.0018) and the 20 mg/kg raxibacumab group (p = 0.0036) compared with the placebo group.
- In all subgroups of monkeys confirmed to be bacteremic and/or toxemic at or before the time of treatment initiation, there was a statistically significant survival benefit associated with 20 or 40 mg/kg raxibacumab relative to placebo, with the 40 mg/kg dose resulting in the highest survival rates.
- Survival time was significantly longer in the 20 mg/kg raxibacumab (median > 28 days, p = 0.0029) and 40 mg/kg (median > 28 days, p = 0.0004) groups compared with placebo group (median = 3.3 days).

Other Observations

- Monkeys in this study generally experienced outward clinical signs consistent with inhalation anthrax. Gross findings at necropsy were consistent with death due to inhalation anthrax and histopathology revealed microscopic lesions typical of inhalation anthrax.
- All monkeys found dead or that were euthanized were positive for bacteremia on terminal blood culture.
- Surviving monkeys developed positive TNA titers by Day 28 post challenge.

13.11 Appendix 11: Raxibacumab/Antibiotic Combination Efficacy Studies

13.11.1 Pilot Study of Levofloxacin as Therapeutic Treatment in Rabbits with Inhalation Anthrax (Study 723-G005835)

This non-GLP study, "Evaluation of Levofloxacin for Post-exposure Treatment in the NZW Rabbit Inhalation Anthrax Model," evaluated the efficacy of different levofloxacin dose levels when administered orally for 3 consecutive days as a therapeutic treatment against lethality due to inhalation exposure to *B. anthracis* in rabbits. The study also evaluated plasma PK parameters to determine which levofloxacin dose regimen achieved systemic/plasma PK exposure in animals sufficiently similar to the exposure achieved in humans at the clinically relevant doses. The results of this pilot study were used to support the dose selection for Study 781-G923701.

Study Design and Analysis

This was an open-label, parallel-group, randomized, and controlled study to evaluate the efficacy of different levofloxacin dose levels when administered orally once daily for 3 consecutive days as a therapeutic treatment against lethality due to inhalation exposure to *B. anthracis*.

Twenty-four NZW rabbits were randomized by gender and body weight into each of 3 treatment groups (10 mg/kg, 25 mg/kg, and 50 mg/kg levofloxacin, Levaquin® Oral Solution) with 8 rabbits per active treatment group, 50% males and 50% females); 3 additional animals (all male) received no study agent and served as controls to ensure lethality of the challenge. All rabbits were to be challenged with a target 200 x LD_{50} dose of *B. anthracis* (Ames strain) spores. The trigger for treatment of individual rabbits with levofloxacin was 1st rise in body temperature at 2 consecutive time points after aerosol challenge equal to 2 standard deviations (or minimum 1.5°F) higher than its baseline pre-challenge average temperature. For each rabbit in an active treatment group, 1 dose of levofloxacin was administered by oral gavage daily for 3 days.

The parameters to be measured included clinical observations, temperature, serum PA, and bacteremia.

Eight animals per group were considered adequate to assess mortality rates at Day 21 and to evaluate PK.

Because this was an exploratory analysis, no formal efficacy analyses were performed. The percentage of rabbits alive at Study Day 21 and median time to death were calculated for each group. All deaths observed post spore challenge were to be defined as failures.

Results

The rabbits in the treatment groups were challenged with a range of maximum of 231 x LD_{50} to 624 x LD_{50} dose of *B. anthracis* (Ames strain) spores. The average exposure for all treated rabbits was 486 ± 104 x LD_{50}.

The 21-day survival rates are provided in Table 13-19 and Figure 13-23. The survival rates were high in all 3 levofloxacin treatment groups and there were no survivors in the placebo group. There was no dose response in improved survival across the 3 levofloxacin treatment groups.

Table 13-19 Survival at Day 21 (723-G005835)

Treatment	N	Number (%) of Survivors
Control	3	0 (0)
10 mg/kg levofloxacin	8	7 (87.5%)
25 mg/kg levofloxacin	8	5 (62.5%)
50 mg/kg levofloxacin	8	7 (87.5%)

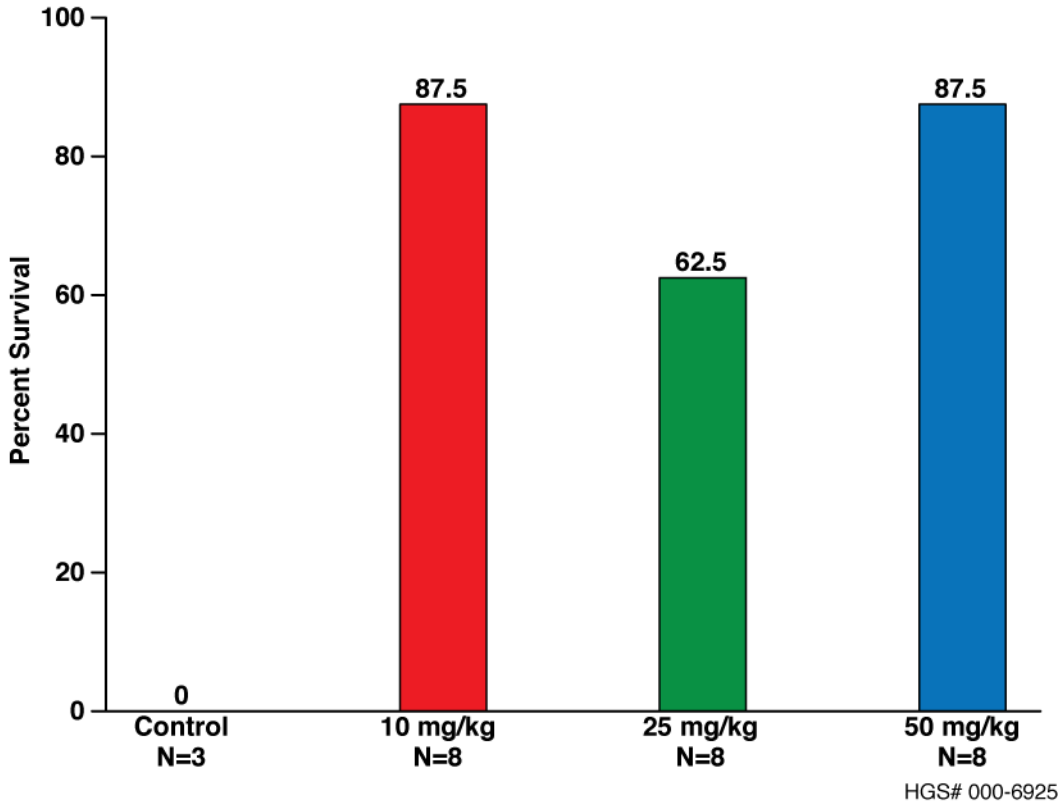

Figure 13-23 Survival at Day 21 (723-G005835)

Because all rabbits received the treatment to which they were randomized, the as-treated population was identical to the ITT population.

The secondary efficacy analysis was survival time, defined as the time from spore challenge to death during the 21-day period. The mean survival time in the 8 animals that died on study were 63.5 (n = 3), 45.2 (n = 1), 262.0 (n = 3), and 84.2 (n = 1) hours for the control, 10, 25, and 50 mg/kg levofloxacin groups, respectively (Figure 13-24). There was no apparent dose response in survival time. Because less than half of the animals died in each of the levofloxacin-treated groups, no median survival time could be calculated for these groups. The median survival time in the control group was 2.9 days.

Of the 24 rabbits who received active treatment, 22 were bacteremic at the time of treatment. All 3 control rabbits were bacteremic prior to death. Among the animals that were bacteremic at the time of treatment, levofloxacin treatment sterilized the bacteria within 23.75 hours in all but 1 rabbit and all rabbits were negative for bacteremia by 47.75 hours post levofloxacin treatment.

The 3 control animals and the single animal that died in each of the 10 mg/kg and 50 mg/kg group, succumbed within 4 days of spore challenge. In the 25 mg/kg group, none of the 3 non-surviving rabbits died before 8 days post challenge. One of these rabbits had been bacteremic and toxemic at the time of levofloxacin treatment and lived 9.94 days; 2 of these animals were not bacteremic or toxemic at the time of treatment, although they did exhibit increased temperature that triggered treatment. One animal became bacteremic and toxemic as evidenced by its 8.1-day blood sample and died at 8.95 days. The other animal became toxemic on Day 10 and died at 13.86 days, but did not develop measurable bacteremia at any time while on study. This suggests that the antibiotic treatment regimen in these 2 animals was effective at suppressing bacteremia during the 3-day treatment period, but allowed the emergence of disease after antibiotic treatment was stopped.

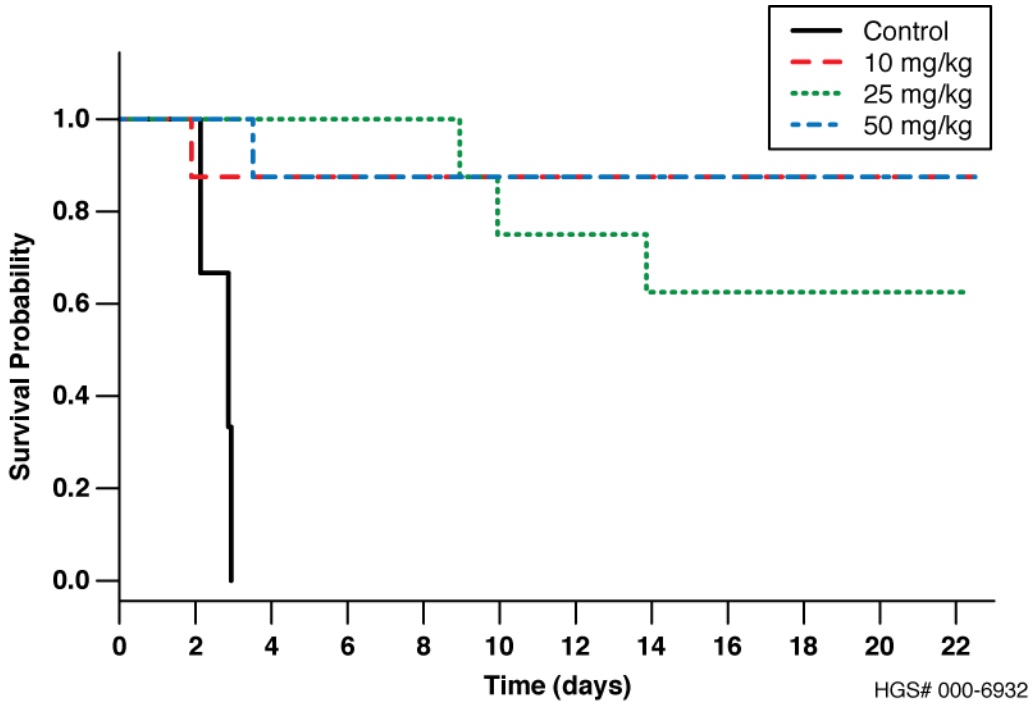

Figure 13-24 Survival curves (723-G005835)

Conclusions

- Levofloxacin at all 3 doses provided a high survival benefit, but there was no apparent dose response across the 3 doses evaluated.
- There was no dose-dependent increase in mean time to death in the non-survivors.
- All 3 levofloxacin dosing regimens resulted in plasma drug concentrations greater than the reported MIC for *B. anthracis* for the duration of each of the 3 consecutive dosing intervals in this study.
- Levofloxacin exposure appears to be proportional to dose across the 5-fold dose range tested, with no difference between genders.
- The 50 mg/kg levofloxacin dose in rabbits produces similar exposure to that reported for the approved human doses of 500 or 750 mg levofloxacin.

13.11.2 Efficacy of Therapeutic Treatment with Levofloxacin in Combination with Raxibacumab in the Rabbit (Study 781-G923701)

This GLP study, "Evaluating the Efficacy of Raxibacumab in Combination with Levofloxacin for Post-exposure Treatment in the New Zealand White Rabbit Inhalational Anthrax Model," evaluated the efficacy of raxibacumab in combination with levofloxacin for therapeutic treatment in the NZW rabbit inhalational anthrax model.

Study Design and Analysis

This was a blinded, parallel-group, randomized, placebo-controlled GLP study to evaluate the therapeutic efficacy of 50 mg/kg levofloxacin administered once daily by oral gavage for 3 days with or without concomitant administration of a single IV dose of 40 mg/kg raxibacumab in anthrax spore inhalation-challenged rabbits experiencing symptoms of inhalation anthrax. Per discussion with the Agency, the antibiotic regimen chosen for the raxibacumab/antibiotic combination studies was to have been adequate to demonstrate sterilization of bacteremia in the animals. The levofloxacin pilot study (723-G005835) demonstrated that bacteria were sterilized in many anthrax-challenged animals by 24 hours and in all animals by 3 days.

There were 3 treatments groups in the study: raxibacumab (single IV 40 mg/kg dose) in combination with levofloxacin (50 mg/kg administered every 24 hours x 3), levofloxacin alone, and placebo control. In total, 52 rabbits were studied: 12 rabbits in the placebo arm, 20 rabbits in the levofloxacin alone arm, and 20 rabbits in the levofloxacin/raxibacumab combination arm. All rabbits were to be challenged with a target 200 x LD_{50} dose of *B. anthracis* (Ames strain) spores. Treatment with raxibacumab and with levofloxacin was initiated based on individual onset of detectable serum PA or increased body temperature, consistent with the criteria for therapeutic intervention used in the pivotal rabbit efficacy study (682-G005758).

The study design provides approximately 99% power at the 5% significance level to detect an absolute improvement of 71.7% or more in the 28-day survival in the raxibacumab/ levofloxacin combination arm, based on the assumption of 8.3% (1 of 12) survival at Day 28 in the placebo control group and 80% (16 of 20) survival in the combination arm.

The primary efficacy endpoint was survival at Day 28 in the ITT population. The study period was longer in this study than in the pivotal rabbit efficacy study (Study 682-G005758) to allow for observation of potential late death due to reemergence of bacteremia after the pressure of antibiotics was removed. Animals that were spore-challenged, but died before receiving placebo or any active treatment, were to be included in this population as treatment failures. The primary analysis of the primary efficacy endpoint was to compare the percent of animals alive at study Day 28 post challenge between the placebo control group and the levofloxacin/raxibacumab combination treatment group. The comparison was to be performed using the 2-sided Fisher's exact test. The survival rates were also compared between the levofloxacin group and the placebo group and between the 2 levofloxacin treatment groups. Survival time and subgroups analyses were also to be performed.

In correspondence from the Agency dated 17 November 2006 it was advised that demonstration of the superiority of raxibacumab over placebo can be the basis for licensure. Because raxibacumab will likely be used in combination with antimicrobials, animal studies to evaluate the efficacy of raxibacumab with antimicrobial are important to assess the possible antagonism between raxibacumab and antimicrobial and/or the possible benefit of raxibacumab/antimicrobial compared with antimicrobial alone. The superiority of

raxibacumab over placebo has been demonstrated in rabbits and monkeys and thus, the purpose of this raxibacumab/levofloxacin study was to assess possible antagonism or benefit of the combination, rather than to show superiority over antimicrobial alone. Further, it should be noted that this study could not be adequately powered to statistically evaluate a survival benefit of the raxibacumab/levofloxacin combination arm over levofloxacin alone due to limitations on the number of rabbits that can ethically and logistically be included in a study. For example, the sample size to yield 80% power at the 5% significance level to detect a 15% greater survival benefit of raxibacumab/antimicrobial (95%) vs antimicrobial alone (80%) would require 81 animals per group.

The parameters measured included clinical observations, hematology, temperature, serum PA, bacteremia, TNA, and MIC. Gross necropsy and histopathology were performed on all animals.

Results

Mean anthrax spore exposure was 293.5 ± 83.6 x LD_{50} and was similar across the treatment groups ($p = 0.3411$). The primary efficacy endpoint was met with statistically higher 28-day survival in the levofloxacin/raxibacumab (19/20, 95%, $p < 0.0001$) and the levofloxacin group (19/20, 95%, $p < 0.0001$) vs placebo (0%) as shown in Table 13-20 and Figure 13-25. There was no difference in survival rates between the 2 active treatment arms.

Table 13-20 Survival at Day 28 (781-G923701)

Treatment	N	Number (%) of Survivors	P-value vs Control[1]
Control	12	0 (0%)	
Levofloxacin	20	19 (95%)	< 0.0001
Raxibacumab/Levofloxacin[2]	20	19 (95%)	< 0.0001

[1] From 2-sided Fisher's exact test for the comparison vs the control group.

[2] Difference in % survivors (95% CI) is 0% (-20.1%, 20.3%) between the raxibacumab/levofloxacin vs levofloxacin alone groups.

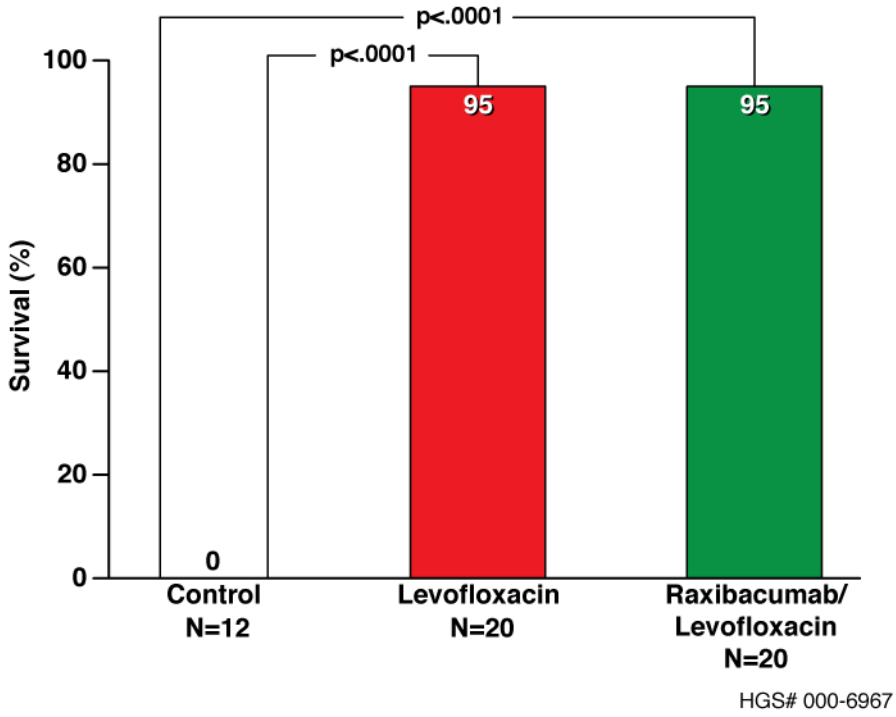

Figure 13-25 Survival at Day 28 (781-G923701)

The 1 death in the raxibacumab/levofloxacin combination group was attributed to complications of an incorrect gavage and was not attributed to anthrax since there was no evidence of bacteremia. Excluding this animal from the efficacy analysis, there was a 100% survival rate in the raxibacumab/levofloxacin combination group.

Because all rabbits received the treatment to which they were randomized, the as-treated population is identical to the ITT population.

Several subgroups by bacteremia and toxemia status were prespecified. Figure 13-26 displays the results for the prespecifed subgroups of animals and demonstrates that the survival benefit in all prespecified subgroups is consistent with the statistically significant effect observed in the overall ITT population. In all subgroups, the confidence intervals contained the point estimate of Day 28 survival in the raxibacumab/levofloxacin group, indicating that the survival benefits between the levofloxacin group and the raxibacumab/levofloxacin group were not significantly different.

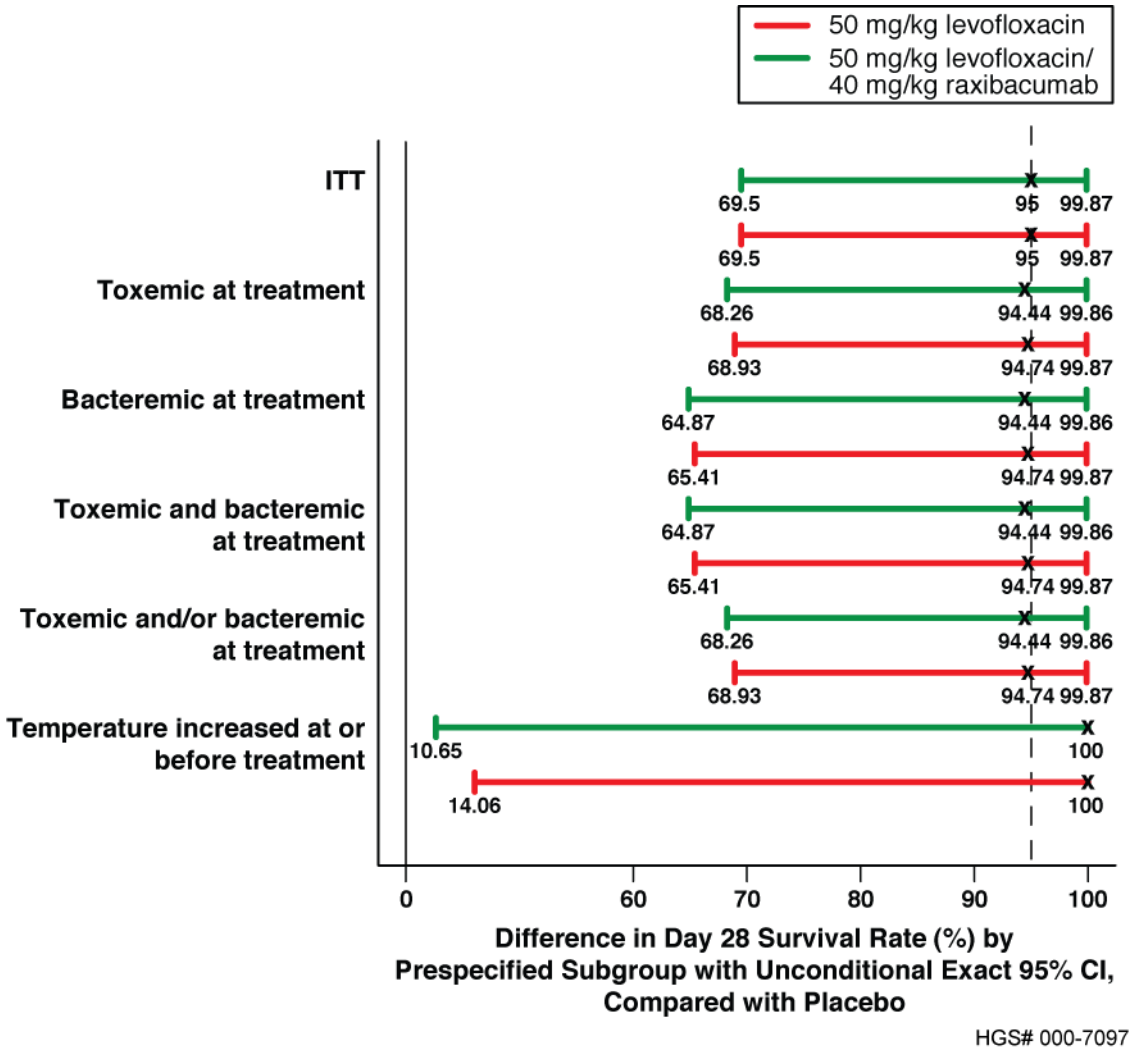

Figure 13-26 **Absolute improvement and unconditional exact 95% CI in Day 28 survival compared with placebo by subgroup of the ITT population (781-G923701)**

As shown in Figure 13-27, survival time, the secondary efficacy endpoint, was significantly longer in the ciprofloxacin group ($p < 0.0001$) and the levofloxacin/raxibacumab group ($p < 0.0001$) relative to the placebo group. The difference in survival times between the 2 active treatment groups was not statistically significant ($p = 0.9855$ as determined by log-rank test). The median survival time in the placebo group was 3.3 days. Median survival time could not be determined for the levofloxacin and levofloxacin/raxibacumab groups because the medians extended beyond 28 days.

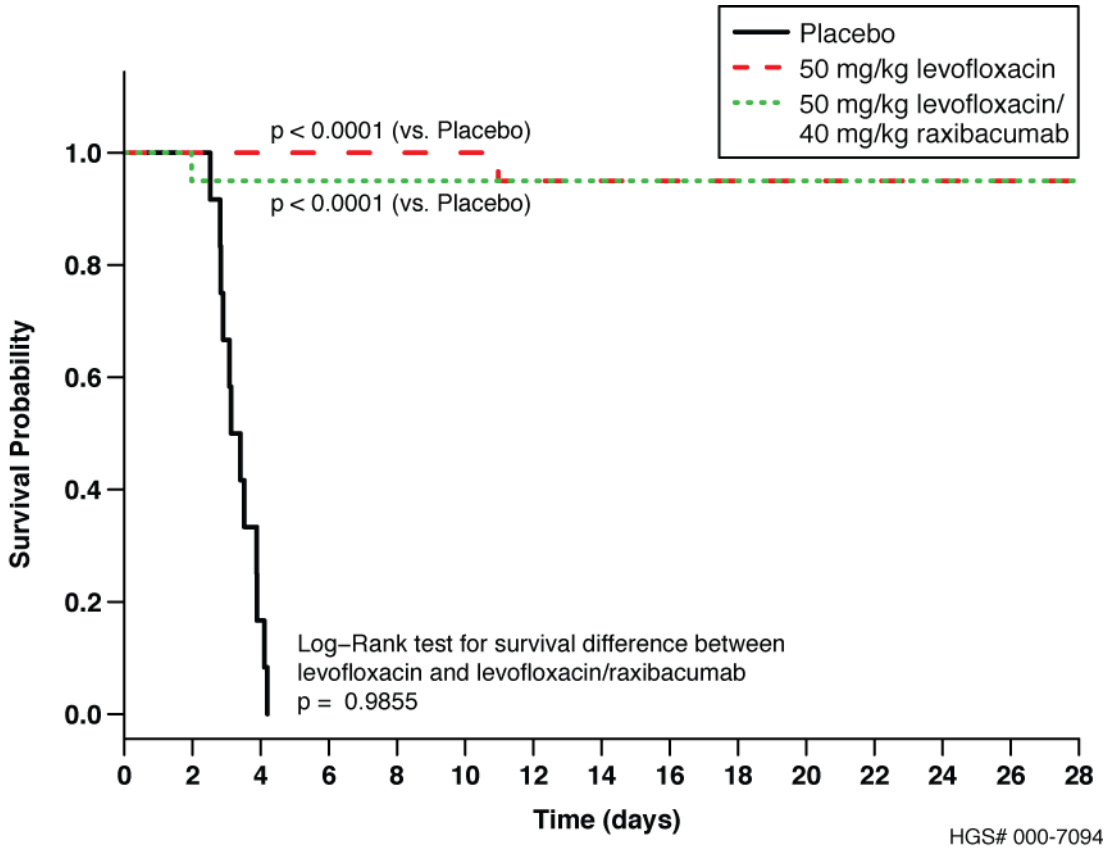

Figure 13-27 Survival curves (781-G923701)

In total, the bacteremia results confirmed that 47 of 52 animals (90%) had active anthrax prior to treatment: 10 animals in the placebo group (83%), 19 animals in the levofloxacin group (95%), and 18 animals in the levofloxacin/raxibacumab group (90%). Although 2 animals in the placebo group did not have a positive culture prior to challenge, positive blood cultures were documented in both animals after treatment and up until time of death.

PA levels over time by individual rabbit are provided for placebo-treated rabbits (all non-survivors), levofloxacin-treated survivors and 1 non-survivor, and levofloxacin/raxibacumab-treated survivors and 1 non-survivor in Figure 13-28. All rabbits tested negative for PA toxemia prior to challenge. PA values rose sharply by 24 hours post challenge. Among placebo-treated rabbits, PA values exhibited a slight plateau during the period from 24 to 48 hours post challenge followed by a 2nd rapid increase until death. There were no plateaus of PA levels among levofloxacin and levofloxacin/raxibacumab-treated survivors; rather a rapid decline in PA levels in the majority of rabbits followed the initial rise and continued until the end of the study. The magnitude of the PA increase in the 1 levofloxacin-treated non-survivor was far less than among the majority of rabbits in the study, and PA declined to baseline by 72 hours post challenge. This was followed by a late period of PA increase starting 7 days post challenge (and 2 days post termination of antibiotic

dosing) and continuing until death. This rabbit had its' treatment triggered by PA toxemia at 24.5 hours post challenge and became bacteremic; however, the animal did not develop elevated body temperature prior to death. The 1 non-survivor in the levofloxacin/raxibacumab group did not display a plateau in PA levels after the initial rise; rather the PA levels declined until death.

Fifteen of 18 rabbits (83%) in the levofloxacin/raxibacumab group tested potentially positive for anti-raxibacumab antibodies by Day 28. In addition, 6/19 (31.6%) rabbits in the levofloxacin group tested potentially positive for anti-raxibacumab antibodies on Day 28. Although the levofloxacin-only treatment group did not receive raxibacumab, the levofloxacin-treated rabbits mounted an anti-PA response which may have included antibodies that cross-reacted in the anti-human anti-PA assay. Given the modest incidence of anti-raxibacumab immunogenicity at low titers compared with the raxibacumab-treated animals, this explanation is plausible. In any case, emergence of potential anti-raxibacumab immunogenicity did not negatively impact survival.

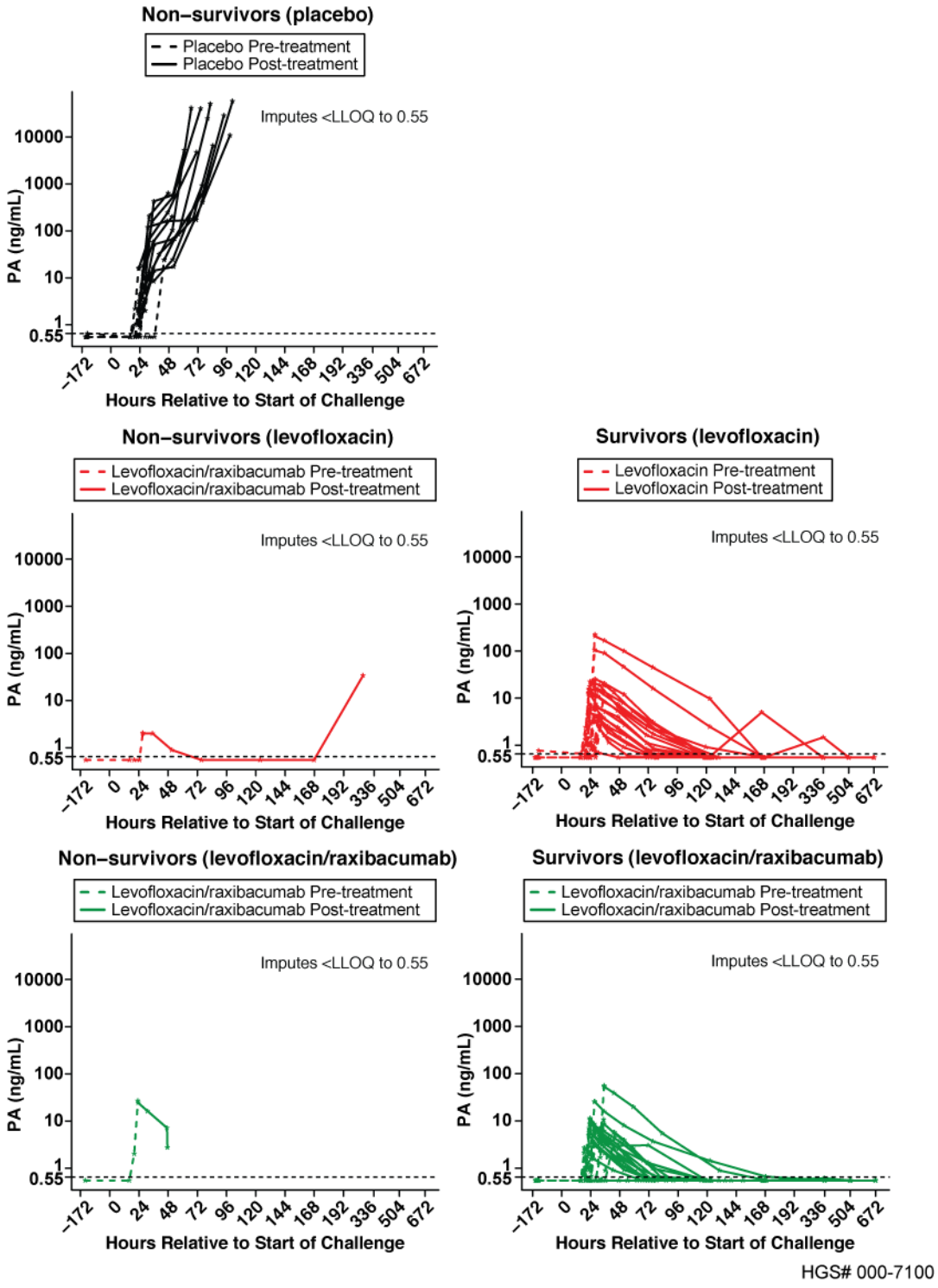

Figure 13-28 PA levels over time by individual rabbit (781-G923701)

Gross necropsy and microscopic examination were conducted on all 52 animals, survivors and non-survivors. Few gross lesions were evident in rabbits surviving to study termination, and these lesions did not correlate histologically with evidence of anthrax. Gross lesions in rabbits dying on study included enlargement, discoloration and/or foci of the adrenal glands, appendix, brain, lung and multiple lymph nodes; fluid (effusion) in the pericardial and thoracic cavities, and fluid/thickening (edema) of the skin and thymus. These gross lesions are typical of anthrax in rabbits and correlated histologically with necrosis, inflammation, hemorrhage, edema and anthrax bacteria. Lung discoloration in the only animal in the levofloxacin/raxibacumab group to die spontaneously (K99246) did not correlate with microscopic evidence of anthrax; the death was attributed to a gavage accident. Microscopic findings consistent with anthrax were present in all rabbits dying spontaneously or becoming moribund following anthrax challenge except for animal K99246, which was not bacteremic at death. None of the rabbits in the levofloxacin and levofloxacin/raxibacumab treatment groups had brain lesions on microscopic examination, although hemorrhage and meningitis were noted in 1 placebo-treated animal. None of the rabbits surviving to study termination had lesions attributable to anthrax at sacrifice.

The MIC results confirmed that bacteria cultured from the blood of challenged animals were inhibited by similar concentrations of levofloxacin and ciprofloxacin as the challenge material.

Conclusions

- The primary efficacy endpoint was met, with statistically higher 28-day survival in the levofloxacin/raxibacumab group (19/20, 95.0%, $p < 0.0001$) relative to placebo (0%); the result was same for the levofloxacin treatment group (95% survival, $p < 0.0001$ compared with placebo (0%).
- In both the as-treated and the anthrax deaths populations, the survival benefits between the levofloxacin group and the levofloxacin/raxibacumab group were not statistically significantly different.
- The survival benefit in all prespecified subgroups was consistent with the statistically significant effect observed in the overall ITT population.
- Survival time was significantly longer in the levofloxacin group ($p < 0.0001$) and the levofloxacin/raxibacumab group ($p < 0.0001$) relative to the placebo group. The difference in survival times between the 2 active treatment groups was not statistically significant ($p = 0.9855$ as determined by log-rank test).
- The emergence of anti-raxibacumab immunogenicity, which was expected, and higher in incidence and degree in the levofloxacin/raxibacumab group compared to the levofloxacin group, did not affect survival.
- Rabbits in this study generally experienced outward clinical signs and altered hematology and CRP consistent with anthrax infection, and those that succumbed to disease followed the hallmark progression pattern characteristic of inhalation anthrax.
- All survivors were negative for bacteremia and none had evidence of anthrax-related pathology by microscopic examination at sacrifice.

- Co-administration of IV raxibacumab had no effect on levofloxacin exposure for IG levofloxacin doses.

13.11.3 Efficacy of Therapeutic Treatment with Ciprofloxacin in Combination with Raxibacumab in the Monkey (Study 789-G923702)

This GLP study, "Evaluation of the Efficacy of Raxibacumab in Combination with Ciprofloxacin for Therapeutic Treatment in the Cynomolgus Monkey Inhalation Anthrax Model," evaluated the efficacy of raxibacumab in combination with ciprofloxacin for therapeutic treatment in the cynomolgus monkey inhalational anthrax model.

Study Design and Analysis

This was a blinded, parallel-group, randomized, placebo-controlled study to evaluate the therapeutic efficacy of 75 mg ciprofloxacin administered once daily by oral gavage for 3 days with or without concomitant administration of a single IV dose of 40 mg/kg raxibacumab in anthrax spore inhalation-challenged monkeys experiencing symptoms of inhalation anthrax.

There were 3 treatments groups in the study: raxibacumab (single IV 40 mg/kg dose) in combination with ciprofloxacin (75 mg administered every 12 hours x 6), ciprofloxacin alone, and placebo control. In total, 40 monkeys were studied: 12 monkeys in the placebo arm, 14 monkeys in the ciprofloxacin alone arm, and 14 monkeys in the ciprofloxacin/ raxibacumab combination with arm. All monkeys were to be challenged with a target 200 x LD_{50} dose of *B. anthracis* (Ames strain) spores. Treatment with raxibacumab and with ciprofloxacin was initiated based on individual onset of detectable serum PA. All monkeys received a single 1 mg/kg IM dose of diphenhydramine prior to raxibacumab/placebo administration as they did in the monkey efficacy study (724-G005829) to reflect premedication with diphenhydramine in the human clinical trials.

This study design provided approximately 85% power at a 5% overall significance level to detect an absolute improvement of 56% or more survival at Day 28 post challenge in the ciprofloxacin/raxibacumab combination group. The sample size calculation assumed an 8.3% (1 of 12) survival at Day 28 in the placebo control group and a 64.3% (9 of 14) survival at Day 28 in the ciprofloxacin/raxibacumab combination group.

The primary efficacy endpoint was survival at Day 28 in the ITT population. Animals that were spore-challenged, but died before receiving placebo or any active treatment, were to be included in this population as treatment failures. The primary analysis of the primary efficacy endpoint was to compare the percent of animals alive at study Day 28 post challenge between the placebo control group and the ciprofloxacin/raxibacumab combination treatment group. The comparison was to be performed using the 2-sided Fisher's exact test. The survival rates were also compared between the ciprofloxacin group and the placebo group and between the 2 ciprofloxacin treatment groups. Survival time and subgroups analyses were also to be performed.

The parameters measured included clinical observations, hematology temperature, serum PA, bacteremia, TNA, and MIC.

Results

In total, 40 monkeys were screened, randomized and challenged with anthrax spores (12 in the placebo group and 14 in each of the ciprofloxacin and ciprofloxacin/ raxibacumab groups). Mean anthrax spore exposure was 275.7 ± 87.0 x LD_{50} and was not significantly different ($p = 0.0688$) across the treatment groups: 228 x LD_{50}, 292 x LD_{50}, and 302 x LD_{50} in the placebo, the ciprofloxacin, and the ciprofloxacin/raxibacumab groups, respectively.

Across the 3 treatment groups there were no statistically significant differences in time to treatment, events triggering treatment initiation, or the onset of bacteremia and toxemia. Thirty-eight of 40 (95%) monkeys exhibited positive serum PA levels by 54 hours post challenge as determined by the screening assay. The remaining 2 monkeys tested positive for PA while on study. Thirty-six of 40 (90%) monkeys were bacteremic at or before treatment initiation. Two of the remaining 4 monkeys were bacteremic within 64 hours post challenge, and 2 did not test positive for bacteremia while on study; however, both were toxemic by 54 hours post challenge.

The 28-day survival rates are provided in Table 13-21 and Figure 13-29. The primary efficacy endpoint was met: 28-day survival was significantly higher in the ciprofloxacin/raxibacumab group (12/14, 85.7%, $p < 0.0001$) and the ciprofloxacin group (14/14, 100%, $p < 0.0001$) compared with placebo (0%). The difference in survival rates between the 2 active treatment arms was not statistically significant ($p = 0.4815$ based on Fisher's exact test).

Of note, 1 animal in the ciprofloxacin/raxibacumab was euthanized at 3.7 days post-challenge due to seizure. Upon necropsy, lung discoloration was noted suggesting pneumonia related to a potential gavage error. Histopathologic evaluation was consistent with fibrinosuppurative bronchopneumonia related to gavage error, as no *B. anthracis* organisms were evident in any tissue. In addition, no positive blood cultures were observed in this animal post-treatment. Taken together these results suggest that this animal died as a result of a gavage error (ciprofloxacin deposited into the lungs) and not anthrax. However, this death was counted as a treatment failure in the ITT population. Statistically higher 28-day survival was maintained in the ciprofloxacin group/raxibacumab (12/13, 92.3%, $p < 0.0001$) and the ciprofloxacin group (14/14, 100%, $p < 0.0001$) relative to placebo (0.0%) in the analysis that excluded animal suspected to have died from complications associated with gavage error rather than anthrax disease.

Table 13-21 Survival at Day 28 (789-G923702)

Treatment	N	Number (%) of Survivors	Difference in % Survivors (95% CI)[1]	P-Value[2]
Placebo	12	0 (0.00%)	-	-
Ciprofloxacin	14	14 (100.0%)	100.00 (73.03, 100.00)	< 0.0001
Ciprofloxacin/raxibacumab	14	12 (85.71%)	85.71 (54.97, 98.22)	< 0.0001

[1] Difference in % survivors between each active treatment vs placebo with exact 95% confidence interval.

[2] P-value based on 2-sided Fisher's exact test for the comparison vs placebo group.

Difference of % survivors (95% CI) is -14.29 (-42.81, 11.88) between the 2 active treatment groups (Raxi/Cipro vs Cipro groups). P-value = 0.4815 based on Fisher's exact test for the comparison of the 2 active treatment groups.

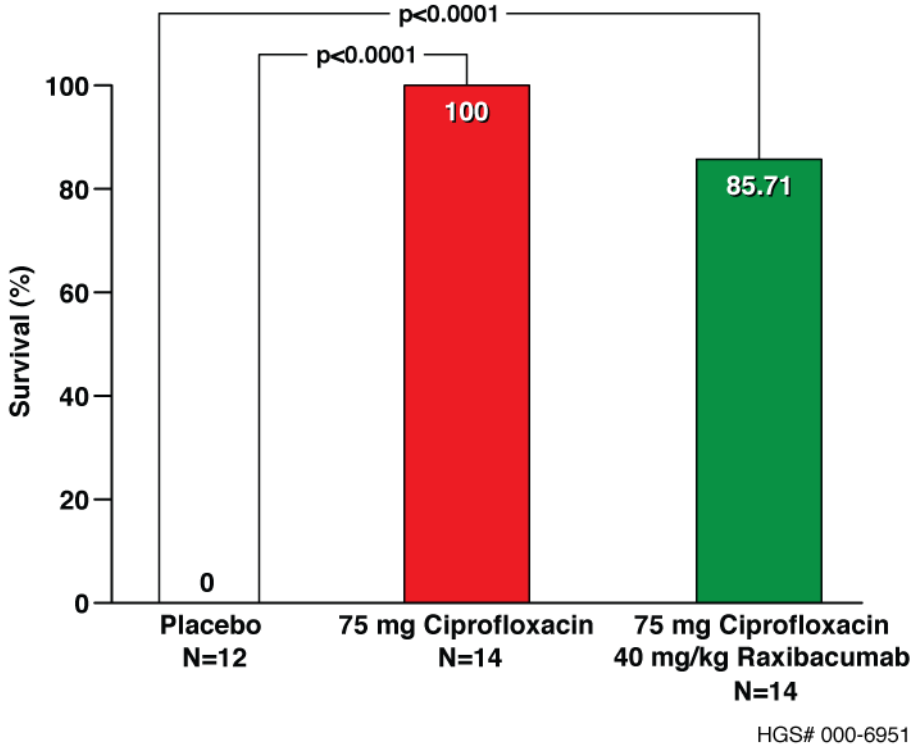

Figure 13-29 Survival at Day 28 (789-G923702)

Because all monkeys received the treatment to which they were randomized, the as-treated population is identical to the ITT population.

Per protocol, monkeys that survived the 28-day study period were to be observed for an additional 60 days. As an exploratory analysis of the primary efficacy endpoint, survival following the 60-day additional observation period was compared among each active treatment group and the placebo group in the ITT population. Percent survival at the

end of the additional 60-day observation period was statistically significantly higher for the ciprofloxacin/raxibacumab group (12/14, 85.7%, p < 0.0001) and the ciprofloxacin group (13/14, 92.9%, p < 0.0001) relative to placebo (0%) and the difference in survival rates between the 2 active treatment arms was not statistically significant (p = 1.0000 based on Fisher's exact test). One monkey in the ciprofloxacin group was euthanized on Day 36 due to a thrombotic lesion in the left inguinal region not likely to be related to anthrax.

Several subgroups by bacteremia and toxemia status were prespecified. Figure 13-30 displays the results for the prespecifed subgroups of animals and demonstrates that the survival benefit in all prespecified subgroups is consistent with the statistically significant effect observed in the overall ITT population. In all subgroups, the confidence intervals contained the point estimate of Day 28 survival in the raxibacumab/ciprofloxacin group, indicating that the survival benefits between the ciprofloxacin group and the raxibacumab/ciprofloxacin group were not significantly different.

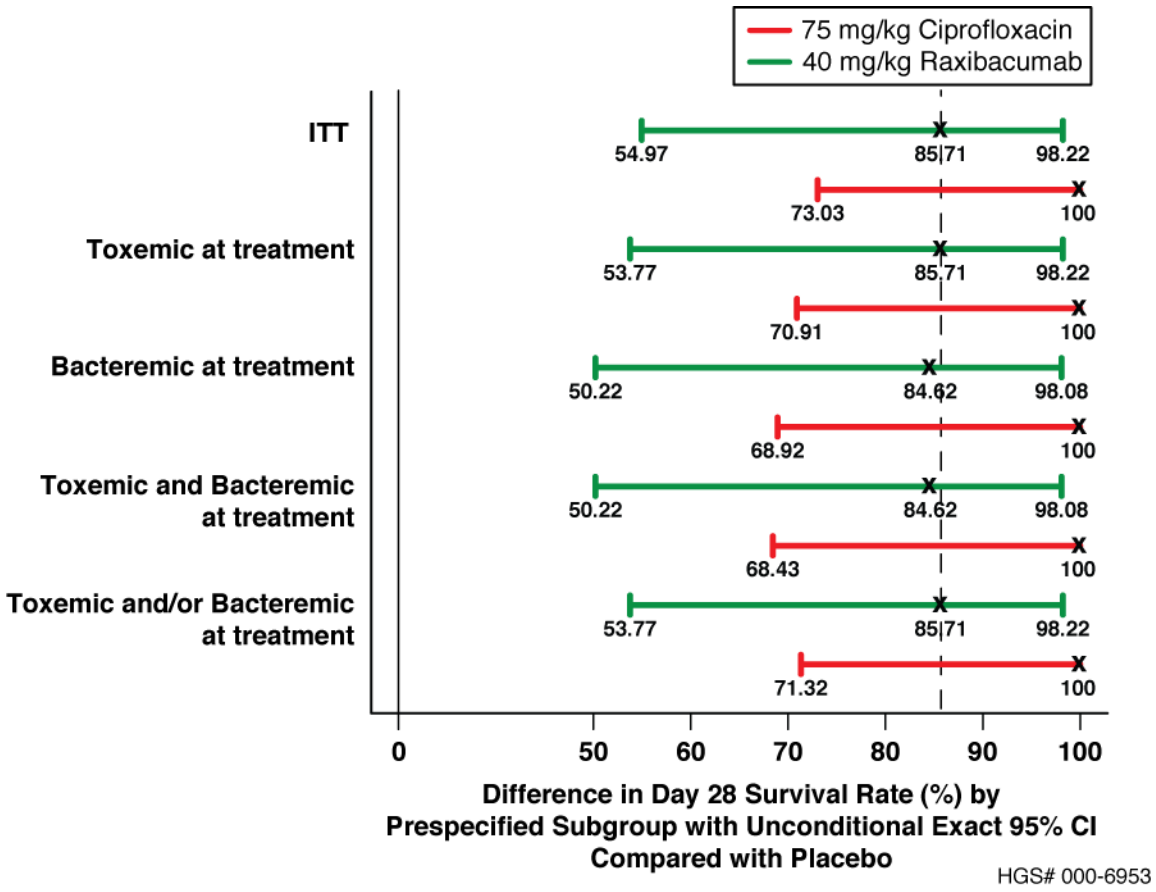

Figure 13-30 **Absolute improvement and unconditional exact 95% CI in Day 28 survival compared with placebo by subgroup of the ITT population (789-G923702)**

As shown in Figure 13-31, survival time, the secondary efficacy endpoint, was significantly longer in the ciprofloxacin group ($p < 0.0001$) and the raxibacumab/ciprofloxacin group ($p < 0.0001$) relative to the placebo group. The difference in survival times between the 2 active treatment groups was not statistically significant ($p = 0.1496$ as determined by log-rank test). The median survival time in the placebo group was 4.2 days. Median survival time could not be determined for the ciprofloxacin and raxibacumab/ciprofloxacin groups because the medians extended beyond 28 days. The same results were obtained in the as-treated population as this population was identical to the ITT.

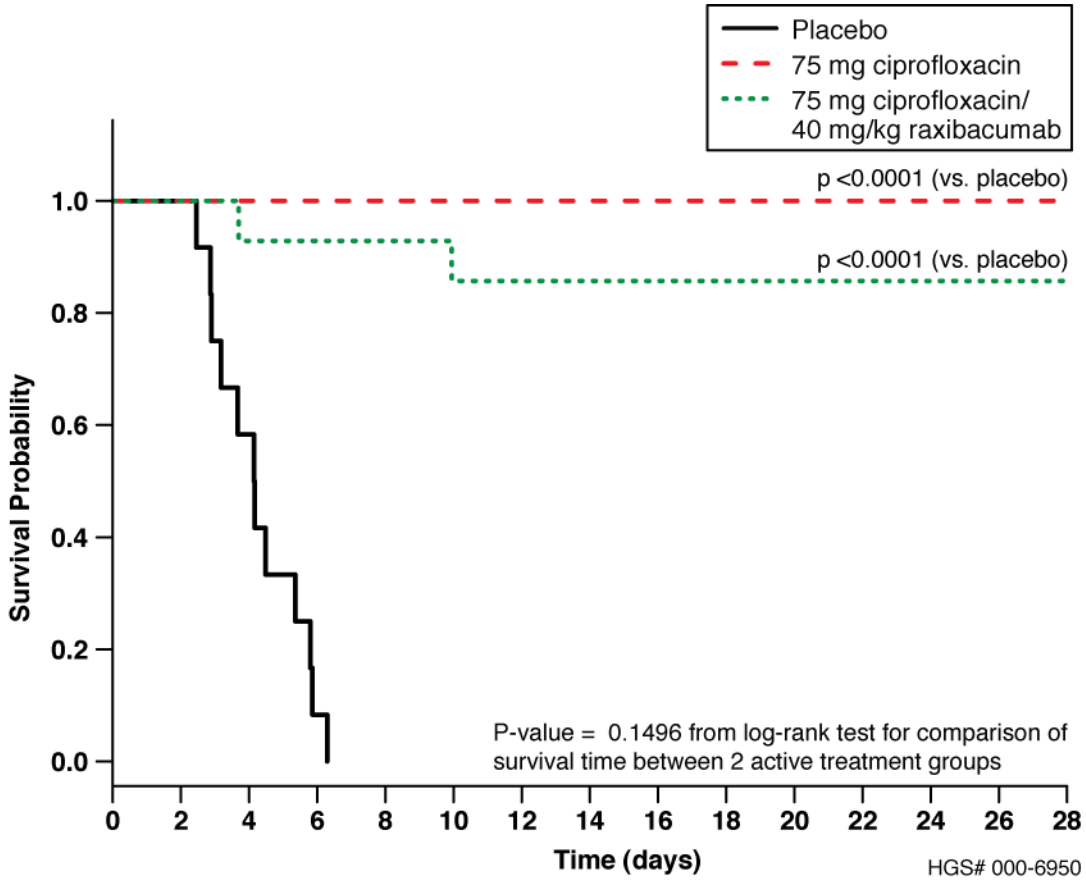

Figure 13-31 Survival curves (789-G923702)

Systemic anthrax infection as evidenced by bacteremia at or before raxibacumab and/or ciprofloxacin treatment was confirmed in 83% of placebo-treated animals and 93% of the animals in each of the ciprofloxacin and ciprofloxacin/raxibacumab-treated groups.

Serum PA levels over time by individual monkey are displayed for placebo-treated monkeys (all non-survivors), ciprofloxacin-treated monkeys (all survivors), and raxibacumab/ ciprofloxacin-treated survivors and non-survivors in Figure 13-32. Serum PA was not detected prior to challenge for any monkey in this study. PA values rose sharply just prior to

40 hours post challenge. Among some placebo-treated non-survivors, PA values exhibited a brief plateau during the period from 40 to 80 hours post challenge, which was generally followed by a 2nd rise in PA levels that lasted until death. There was no plateau among ciprofloxacin/raxibacumab-treated non-survivors, and PA levels declined through time of death; for 1 monkey the decline was followed by a rise in PA just prior to death. Among ciprofloxacin and ciprofloxacin/raxibacumab-treated survivors, the 2nd rise in PA did not occur; rather, the PA declined to undetectable levels for the majority of monkeys by the end of the study.

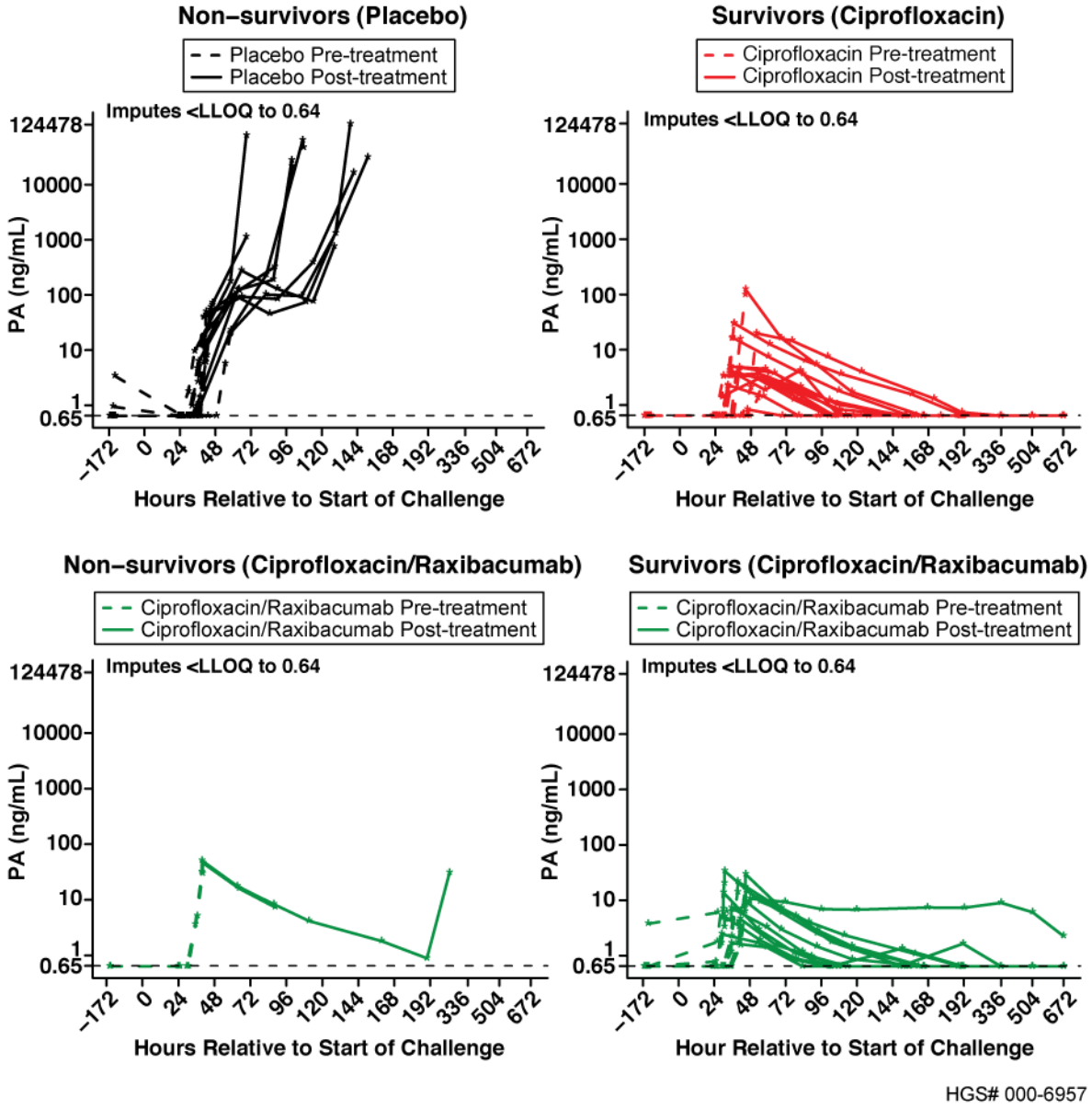

Figure 13-32 PA levels over time by individual monkey (789-G923702)

The serum anti-PA antibody concentration results for the raxibacumab dose groups are summarized in Table 13-22. There were no statistically significant differences in mean serum anti-PA antibody concentrations between the 2 treatment groups, indicating that co-administration of raxibacumab with ciprofloxacin did not affect the formation of anti-PA antibodies by the surviving monkeys, relative to ciprofloxacin alone.

Table 13-22 Serum anti-PA antibody concentrations in surviving monkeys (789-G923702)

		Serum Anti-PA Antibody Concentration (µg/mL)		
		Predose	21 Day Postdose	28 Day Postdose
Ciprofloxacin alone	N	14	14	14
	Mean ± SD	0.068 ± 0.253	65.447 ± 37.687	89.126 ± 48.833
Ciprofloxacin/raxibacumab	N	12	12	12
	Mean ± SD	0.295 ± 0.837	72.506 ± 33.779	100.899 ± 83.862
	P-value[1]	0.3819	0.6222	0.6600

[1] From an unpaired t-test.

The serum TNA titer results are summarized in Table 13-23. The mean serum TNA titers for the ciprofloxacin alone dose group were significantly higher (about 2-fold) than those for the ciprofloxacin/raxibacumab dose group. This difference in mean titers is influenced by a few very high values in the ciprofloxacin group and the median values (Day 21: 2875 and 4761 and Day 28: 3746 and 5585, for ciprofloxacin and ciprofloxacin/raxibacumab, respectively) are not as disparate as the means. It should be noted that all of the surviving monkeys developed TNA titers.

Table 13-23 Serum TNA titers in surviving monkeys (789-G923702)

		Serum TNA Titer		
		Predose	21 Day Postdose	28 Day Postdose
Ciprofloxacin	N	14	14	14
	Mean ± SD	0 ± 0	6369 ± 5288	7266 ± 4864
Ciprofloxacin/raxibacumab	N	12	12	12
	Mean ± SD	0 ± 0	3020 ± 1802	3400 ± 1869
	P-value[1]	-	0.0405	0.0136

[1] From an unpaired t-test.

Gross necropsy and microscopic examination of tissues was performed on all animals that died or were euthanized on study. Gross lesions at necropsy consistent with anthrax included adrenal gland discoloration (indicating the presence of bacteria); brain/meningeal red-stained accumulation, discoloration or foci (hemorrhage and inflammation); abdominal and/or thoracic cavity fluid (effusion); enlargement of axillary, bronchial, mandibular and/or mediastinal lymph nodes (edema, fibrin exudation, and hemorrhage); and skin or thymic fluid

(edema). Morbundity in 1 monkey in the ciprofloxacin/raxibacumab group was consistent with fibrinosuppurative bronchopneumonia related to gavage error, as no *B. anthracis* organisms were evident in any tissue. Microscopic findings consistent with anthrax were present in 13/15 animals examined histologically; the other 2 animals died with lesions unrelated to anthrax. Lesions typical of anthrax in this study included brain hemorrhage and meningitis; tubular degeneration in the kidney; liver necrosis and sinusoidal leukocytosis; edema, fibrin exudation, hemorrhage, neutrophilic inflammation, and lymphocyte necrosis affecting the spleen, thymus, lung and multiple lymph nodes; and the presence of large rod-shaped bacteria consistent with *B. anthracis* in many of the organs evaluated histologically. The only antibiotic-treated monkey to die with lesions of anthrax was animal C31142 (ciprofloxacin/raxibacumab group). This animal had the most severe brain lesions of all animals evaluated histologically but had fewer lesions and bacteria in other organs; time to death for this animal was 10 days, which was more than double the time to death for each of the 4 placebo-treated animals with brain lesions.

The MIC results confirmed that bacteria cultured from the blood of challenged animals were inhibited by similar concentrations of levofloxacin and ciprofloxacin as was the challenge material.

Conclusions

Efficacy

- The primary efficacy endpoint was met, with statistically higher 28-day survival in the ciprofloxacin/raxibacumab group (12/14, 85.7%, $p < 0.0001$) compared with the placebo group (0%). The survival rate in the ciprofloxacin group (14/14, 100%, $p < 0.0001$) was also statistically significantly higher than that of the placebo group. The difference in survival rates between the 2 active treatment arms was not statistically significant ($p = 0.4815$ based on Fisher's exact test).
- The survival benefit in all prespecified subgroups of bacteremia and toxemia at or before treatment is consistent with the effect observed in the overall ITT population. A statistically significant survival benefit was maintained for the raxibacumab/ciprofloxacin and ciprofloxacin groups relative to placebo in all subgroups by bacteremia and toxemia status.
- Survival time was significantly longer in the ciprofloxacin group ($p < 0.0001$) and the raxibacumab/ciprofloxacin group ($p < 0.0001$) relative to the placebo group (all non-survivors). The difference in survival times between the 2 active treatment groups was not statistically significant.
- Across the 3 treatment groups there were no statistically significant differences in anthrax exposure, time to treatment or the onset of bacteremia and toxemia. The groups were also similar with respect to signs and symptoms around the time of treatment, including the number of animals with detectable PA that triggered treatment initiation. The vast majority of monkeys in all groups (35/40, 87.5%) were bacteremic and toxemic at or before treatment initiation.

- Time to PA detection (screening and quantitative), time to bacteremia, and time to treatment were also comparable among survivors and non-survivors across groups.
- Monkeys in this study generally experienced outward clinical signs consistent with inhalation anthrax, and those that succumbed to disease followed the hallmark progression pattern characteristic of inhalation anthrax.
- All surviving monkeys in the ciprofloxacin and raxibacumab/ciprofloxacin groups tested positive at Day 28 for anti-PA antibodies and TNA.

13.12 Appendix 12: Pre-Exposure Prophylaxis and Post-exposure Intervention Efficacy Studies

13.12.1 Pre and Immediate Post-exposure Prophylaxis in the Rabbit (Study 288-HGSIRAB)

This GLP study, "Evaluation of the Prophylactic and Therapeutic Efficacy of HGSI PA Monoclonal Antibody (PA mAb) against Aerosolized Anthrax the Rabbit Model" (288-HGSIRAB) evaluated the prophylactic and post-exposure efficacy of raxibacumab when administered prior to spore challenge and immediately following spore challenge, respectively.

Study Design and Analysis

This was a parallel-group, randomized, placebo-controlled study to evaluate dose-ranging pre-exposure efficacy of raxibacumab. This study comprised 72 naïve rabbits, randomly divided into 6 groups of 12 animals each as follows: Group 1 received vehicle on Study Day -2 and served as the control group; Group 2, Group 3, Group 4 and Group 5 received a single SC dose of raxibacumab at 1, 5, 10 or 20 mg/kg on Study Day –2. Rabbits in Group 6 received a single IV dose of raxibacumab at 40 mg/kg immediately after spore challenge on Day 0. On Study Day 0, all rabbits were exposed to *B. anthracis* spores (Ames strain) at a target dose of 100 x LD_{50}.

The sample size for this study was determined based on the primary efficacy endpoint of survival at Day 14. This study design had 80% power at 5% significance level to detect an absolute improvement of 58.4% or more in the 14-day survival in any 1 of raxibacumab treatment arms. The assumptions for this power calculation were: an 8.3% (1 of 12) survival at Day 14 in the vehicle control group and a 66.7% (8 of 12) survival at Day 14 in any of the raxibacumab-treated groups.

The primary efficacy endpoint for the study was survival at Day 14. The primary analysis of the study was to compare the difference in the survival at Study Day 14 between any 1 of the raxibacumab-treated groups and the vehicle control group using a 2-tailed Fisher's exact test. The Cochran-Armitage test was used to examine the dose response trend of survival at Day 14 among the SC groups.

The secondary endpoint for the study was survival time, defined as the time from spore challenge to death during the 14-day study. The survival time between any 1 of the raxibacumab treatment groups and the vehicle control group was compared using Kaplan-Meier analysis.

The statistical analysis was performed in accordance with the prespecified analytical plan. The primary analysis was performed on the as-treated dataset (which is identical to the intent-to-treat dataset for this study). All deaths were defined as failure in the primary analysis. All statistical tests assume a significance level of 0.05, unless otherwise specified.

In addition to survival rates and time, this study included evaluation of hematology and clinical chemistry parameters and raxibacumab PK.

Results

The mean challenge dose among treatment groups ranged from 174.8 to 242.9 x LD_{50} and there was no significant difference in the inhaled dose among the 6 groups ($p = 0.4802$).

The primary efficacy analysis of the study compared the difference in the survival at Study Day 14 between any 1 of the raxibacumab treatment groups and the vehicle control group. There was a significant difference in the 14-day survival between the vehicle control and raxibacumab treatment groups ($p < 0.0001$, Table 13-24 and Figure 13-33). The 14-day survival was significantly higher in 5 mg/kg SC group (42%) compared with vehicle control group (0%), $p = 0.0373$, as well as all of the higher raxibacumab dose groups: 83%, $p < 0.0001$, was for the 10 and 20 mg/kg SC groups and 100%, $p < 0.0001$, in the 40 mg/kg IV group (100%), $p < 0.0001$.

Table 13-24 Survival at Day 14 (288-HGSIRAB)

Treatment	Number (%) of Survivors	P-value vs Control[1]
Vehicle	0 (0%)	-
1 mg/kg SC, Day -2	0 (0%)	1.000
5 mg/kg SC, Day -2	5 (42%)	0.0373
10 mg/kg SC, Day -2	10 (83%)	< 0.0001
20 mg/kg SC, Day -2	10 (83%)	< 0.0001
40 mg/kg IV, Day 0	12 (100%)	< 0.0001

[1] Obtained from a 2-sided Fisher's exact test. (The p-values for the comparison among all groups are < 0.0001, whether or not the 40 mg/kg IV group was included in the analysis).

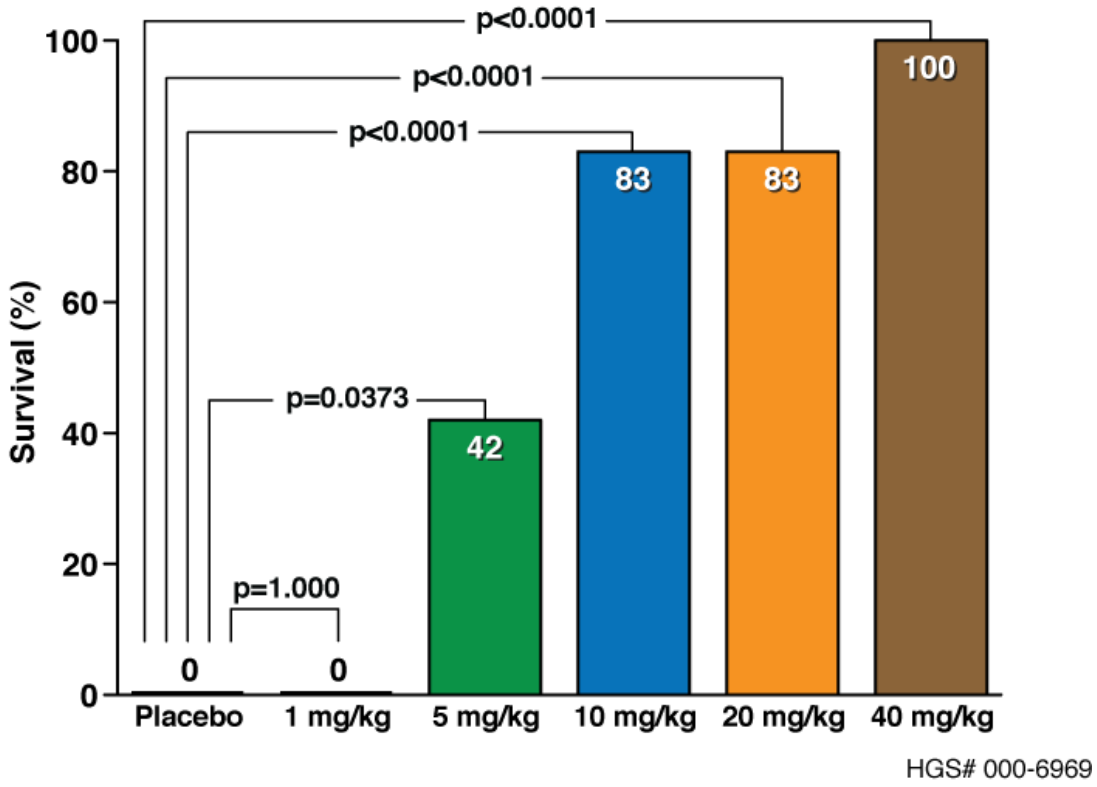

Figure 13-33 Survival at Day 14 (288-HGSIRAB)

The secondary efficacy variable was survival time, defined as the time to death after spore challenge. The survival time of the rabbits was significantly different between the vehicle control and raxibacumab-treated groups ($p < 0.0001$). The median survival time was significantly longer among all the active treatment groups compared to the vehicle control group, all $p \leq 0.0002$ (Figure 13-34). The median survival time was 2 days for the vehicle control group. The median survival time for the 1 mg/kg SC group was 3 days and for the 5 mg/kg SC group was 6.5 days. The median survival time was > 10 days for the 10 mg/kg SC and 20 mg/kg SC groups as well as the 40 mg/kg IV group. There was a significant dose-response trend with respect to the survival at Day 14 ($p < 0.0001$).

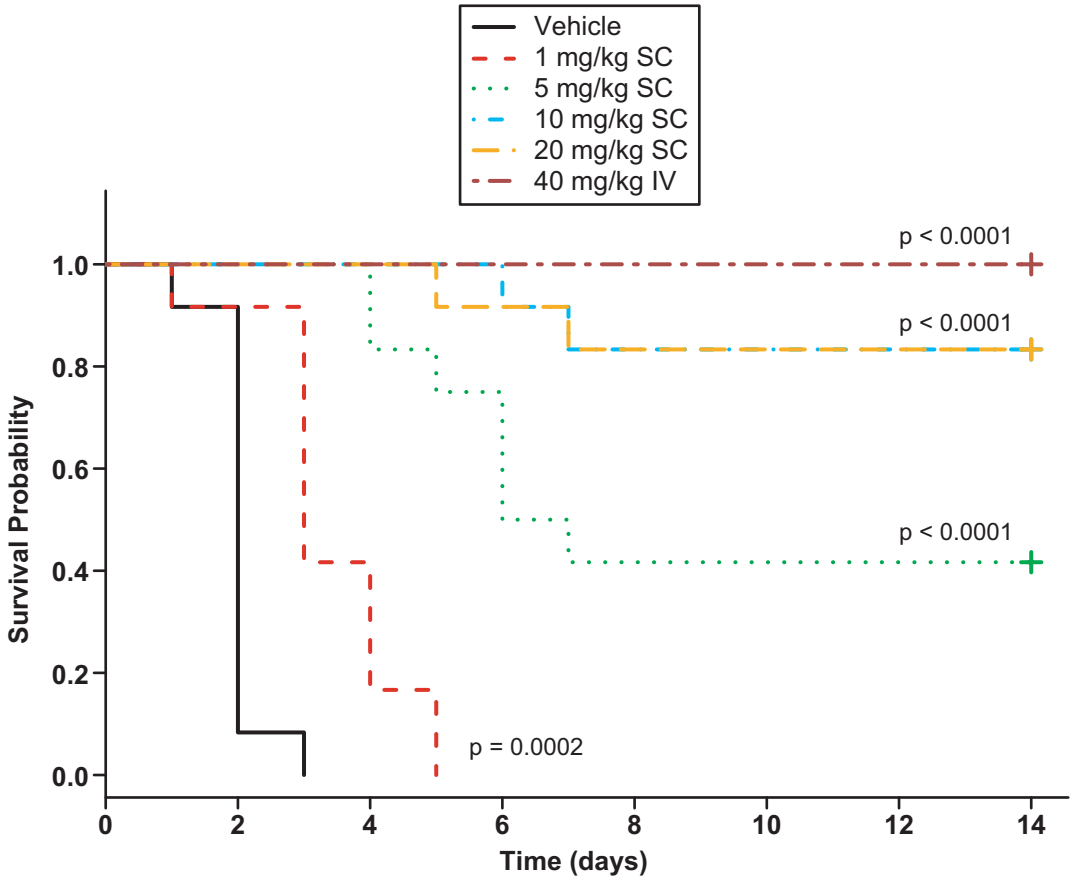

Figure 13-34 Survival curves (288-HGSIRAB)

All p-values were obtained from a log-rank test. The p-values for the comparison among all groups are < 0.0001, regardless of the presence of the 40 mg/kg IV group in the analysis. The p-values marked in the graph are for the comparison vs the vehicle.

There was no significant difference in the 14-day survival or in the time to death between male and female rabbits.

With respect to bacteremia, 1 survivor in the 20 mg/kg raxibacumab treatment group had a positive bacteremia culture on Day 2 and 1 survivor in the 10 mg/kg raxibacumab treatment group had a positive bacteremia culture on Day 7. Both of these rabbits had negative cultures on Day 14. All other cultures for all of the surviving animals through Day 14 were negative. The majority of rabbits that died during the course of the study were bacteremic at death. The presence of bacteremia at Study Day 2 was analyzed using a 2-sided Fisher's exact test. As shown in Table 13-25, bacteremia at Day 2 was significantly reduced in all animals in the raxibacumab treatment groups compared with vehicle ($p < 0.0001$). At Day 2, bacteremia was detected in 11 of the 12 rabbits in the vehicle control group and in only 2 of the 60 raxibacumab-treated rabbits (1 in the 1 mg/kg group and 1 in the 20 mg/kg group). Terminal bacteremia was observed in all animals in the vehicle treatment group and in the

majority of raxibacumab-treated non-surviving animals, where the proportion of animals with terminal bacteremia decreased with increasing raxibacumab dose.

Table 13-25 Bacteremia (288-HGSIRAB)

Treatment/Route	Number (%) with Bacteremia at Day 2	Number (%) with Terminal Bacteremia
Vehicle	11/12 (91.7%)	12/12 (100%)
1 mg/kg SC	1/12 (8.3%)	10/12 (83%)
5 mg/kg SC	0/12 (0%)	5/7 (71%)
10 mg/kg SC	0/12 (0%)	0/2 (0%)
20 mg/kg SC	1/12 (8.3%)	1/2 (50%)
40 mg/kg IV	0/12 (0%)	NA

In the absence of hematology and clinical chemistry data from vehicle control animals at any time point after spore challenge, the relationship of raxibacumab to the hematologic changes could not be determined. The only apparent time-related changes included a reduction in hematocrit (HCT) at Day 7 and an increase in circulating platelets in survivors in all dose groups. Whether these changes reflect a response to spore challenge, raxibacumab, repeated blood sampling, or a combination of these remains unknown. Time-dependent increases in liver enzymes were noted in some animals.

Lesions typical of inhalation anthrax included enlargement of the spleen and lymph nodes (particularly the mediastinal lymph nodes) and hemorrhage and edema in various organs, particularly the meninges, body cavities, skin, and appendix. Gross lesion incidences were largely dose-responsive with greatly reduced numbers of lesions among animals receiving 10 and 20 mg/kg raxibacumab and virtually no lesions present in the rabbits receiving 40 mg/kg.

Conclusions

- Raxibacumab provided significant protection against mortality and prolonged survival time when administered pre-spore exposure or immediately post-spore exposure. This protection was dose-dependent from 1 to 40 mg/kg raxibacumab.
- Raxibacumab significantly reduced bacteremia at all doses compared with vehicle.
- Gross necropsy observations in found dead or euthanized rabbits were consistent with anthrax exposure. The incidence of these findings was inversely proportional to administered dose of raxibacumab.

13.12.2 Post-exposure Intervention in the Rabbit (Study 358-N005999)

This GLP study, "Evaluation of the Prophylactic and Therapeutic Efficacy of HGSI PA Monoclonal Antibody (PA mAb) against Aerosolized Anthrax the Rabbit Model," (358-N005999) examined the efficacy of raxibacumab, when administered IV as a therapeutic treatment at varying time intervals post-spore challenge, against lethality due to inhalational exposure to *B. anthracis.*

Study Design and Analysis

This study comprised 60 naïve NZW rabbits divided into 5 groups of 12 animals (6 males and 6 females) per group. The rabbits were assigned receive a single IV injection of control vehicle (raxibacumab diluent) or 40 mg/kg raxibacumab at 0, 12, 24 or 36 hours post-spore challenge. The target challenge dose was 100 x LD_{50} (Ames strain).

This study design had 80% power at 5% significance level to detect an absolute improvement of 58.4% or more in the 14-day survival in any 1 of the raxibacumab arms. An 8.3% (1 of 12) survival at Day 14 in the vehicle control group and a 66.7% (8 of 12) survival at Day 14 in any of the raxibacumab treatment groups were assumed. Power calculations were performed assuming 2-sided Fisher's exact test for the pairwise comparisons of lethality rates between the vehicle control group and 1 of the raxibacumab treatment groups.

The primary efficacy endpoint for the study was survival at Day 14. The primary analysis of the study was to compare the difference in the survival at Study Day 14 between any 1 of the raxibacumab treatment groups and the vehicle control group using a 2-tailed Fisher's exact test.

The major secondary endpoint for the study was survival time, defined as the time from spore challenge to death during the 14-day study. The survival time of the rabbits that survive at the end of the follow-up was censored at the 14-day study period. The survival time between any 1 of the raxibacumab treatment group and the vehicle control group was compared using Kaplan-Meier analysis.

The statistical analysis was performed in accordance with the prespecified analytical plan. The primary analysis was performed on the as-treated population (which is identical to the ITT population for this study). All deaths were defined as failure in the primary analysis. All statistical tests assume a significance level of 0.05, unless otherwise specified.

In addition to survival rates and time, this study included evaluation of bacteremia.

Results

The mean challenge dose among treatment groups ranged from 65.2 to 128.59 x LD_{50} and there was no significant difference in the inhaled dose among the 6 groups ($p = 0.0684$), although the 24-hour raxibacumab treatment group had the lowest mean spore challenge.

The primary efficacy analysis of the study compared the difference in the survival at Day 14 between any 1 of the raxibacumab treatment groups and the vehicle control group. There was a significant difference in the 14-day survival between the vehicle control and raxibacumab treatment groups ($p < 0.0001$, Table 13-26 and Figure 13-35). The 14-day survival was significantly higher in the 2 raxibacumab groups where the IV injection was administered immediately or 12 hours after challenge (100%), compared with vehicle control group (8.3%), $p < 0.0001$. The 14-day survival rates in the other 2 raxibacumab groups, where the IV

injection was administered 24 hours and 36 hours post spore challenge, were 50% and 41.7%, respectively.

Table 13-26 Survival at Day 14 (358-N005999)

Treatment	Number (%) of Survivors	P-value vs Control[1]
Vehicle	1 (8.3%)	-
Raxibacumab 0 h post challenge	12 (100%)	< 0.0001
Raxibacumab 12 h post challenge	12 (100%)	< 0.0001
Raxibacumab 24 h post challenge	6 (50%)	0.0687
Raxibacumab 36 h post challenge	5 (41.7%)	0.1550

[1] From a 2-sided Fisher's exact test. (The p-values for the comparison among all groups is < 0.0001.)

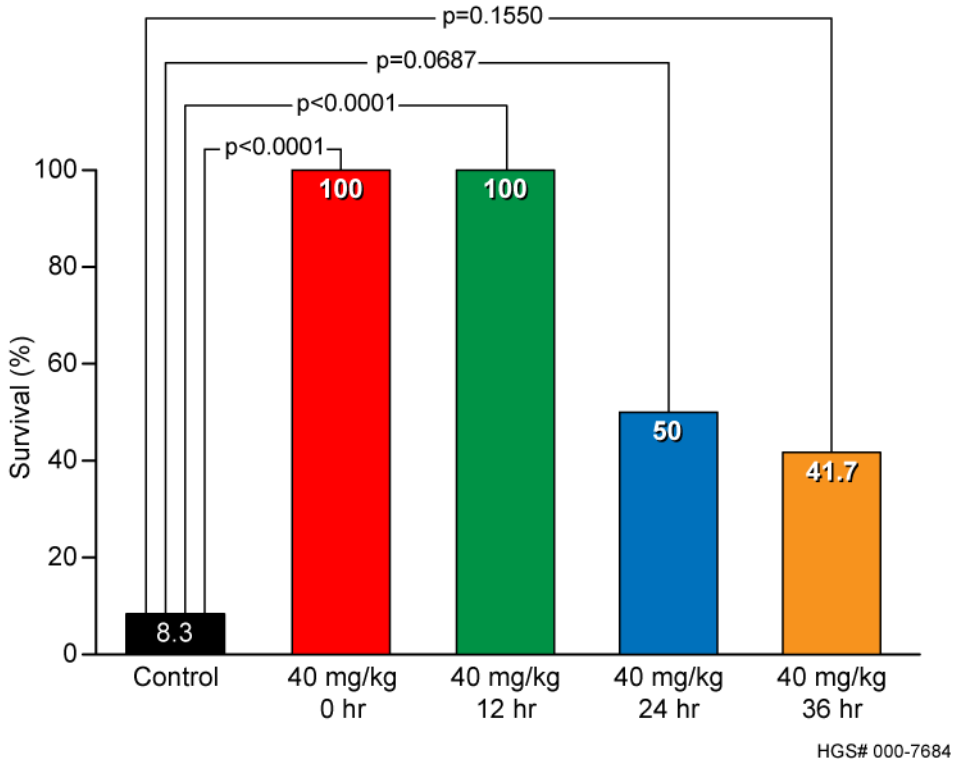

Figure 13-35 Survival at Day 14 (358-N005999)

The secondary efficacy variable was survival time, defined as the time to death after spore challenge. The survival time of the rabbits was significantly different between the vehicle control and raxibacumab treatment groups ($p < 0.0001$, Figure 13-36). All rabbits survived to the end of study when they were treated with raxibacumab immediately or 12 hours after spore challenge. The median survival time was 3 days for the vehicle control group. The median survival time for the 24-hour and 36-hour raxibacumab groups was 8.5 days

(p = 0.0995) and 3.5 days (p = 0.0395), respectively; all animals survived to Day 14 in the 0 and 12 hour raxibacumab groups.

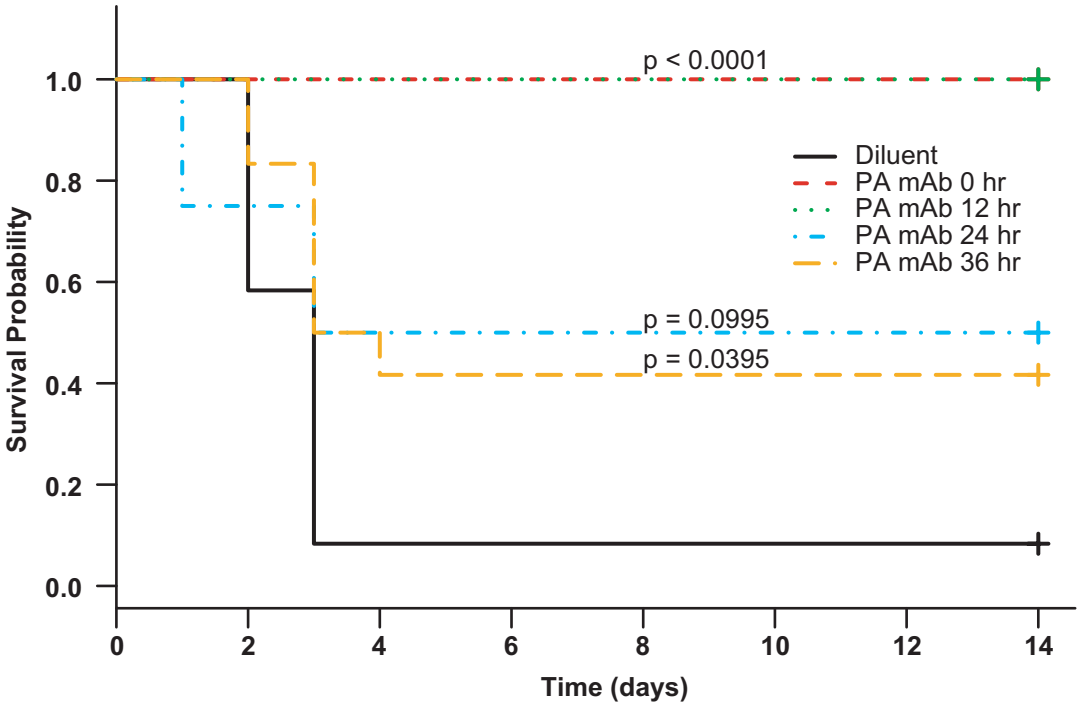

Figure 13-36 Survival curves (358-N005999)

All P-values were obtained from a log-rank test. The p-values for the comparison among all groups are < 0.0001. The p-values marked in the graph are for the comparison vs the vehicle.

Elevations in body temperature occurred primarily 1 to 2 days after spore challenge. In vehicle-treated animals, approximately half of those that died never recorded an elevated temperature. The data are summarized in Table 13-27.

Table 13-27 Body temperature evaluation (358-N005999)

Raxibacumab Intervention Time	Number of Deaths	Incidence of Increased Temperature	Number of Rabbits with Temperature Increase that Died	Number of Rabbits with Temperature Increase that Survived
Vehicle	11/12	6/12	6	0
0 hr	0/12	6/11[1]	0	6
12 hr	0/12	5/11[2]	0	5
24 hr	6/12	6/12	4	2
36 hr	7/12	10/10[3]	7	3

[1] One rabbit was replaced on Day 0 and had no pre-challenge data collected.

[2] One rabbit did not have temperatures recorded due to transponder failure.

[3] Two rabbits had no temperatures recorded due to transponder chip failure.

Bacteremia was 1st observed 24 hours post-exposure, and increased with time to an incidence of 75% by 36 hours after challenge (Table 13-28). Time to death was approximately 1 to 3 days for the majority of rabbits who died in the study. The incidence of terminal bacteremia appeared reduced in those raxibacumab-treated rabbits that did not survive to Day 14. The rabbits that survived were negative for bacteremia on Days 7 and 14.

Table 13-28 Number of rabbits with bacteremia after anthrax spore challenge (358-N005999)

Treatment	Intervention Time (h)[1]	Time Post-Spore Challenge						
		0 h	12 h	24 h	36 h	7 d	14 d	At Death or Euthanasia
Raxibacumab diluent	0	0/12	[2]	[2]	[2]	[3]	[3]	10/11
Raxibacumab	0	0/12	[2]	[2]	[2]	0/12	0/12	0/12
Raxibacumab	12	[2]	0/12	[2]	[2]	0/12	0/12	0/12
Raxibacumab	24	[2]	[2]	1/12	[2]	0/6	0/6	4/6[4]
Raxibacumab	36	[2]	[2]	[2]	9/12	0/5	0/5	3/7

[1] Time following anthrax spore-challenge.

[2] Blood not collected at this time point for this treatment group.

[3] All animals dead by this interval.

[4] One of these animals died after raxibacumab treatment on Day 1 and was positive for bacteremia at that time.

Conclusions

- A single IV dose of 40 mg/kg raxibacumab administered immediately or within 12 hours post-spore challenge provided 100% protection against anthrax-mediated lethality.
- Intervention at 24 hours and 36 hours post-spore challenge provided 50% and 41.7% survival, respectively.
- Increased body temperature was noted in some animals 1 day after spore challenge.
- Bacteremia incidence increased between 24 and 36 hours from 16% to 75%.

13.12.3 Post-exposure Intervention in the Rabbit (Study 371-N006101)

This GLP study, "Post-exposure Therapeutic Intervention with PA mAb in the New Zealand White Rabbit: Dose-response Study" (371-N006101) examined the survival benefit at Day 14 provided by IV administration of raxibacumab as a therapeutic intervention in rabbits exposed by inhalation to *B. anthracis*. This study examined the dose-response relationship of raxibacumab therapeutic intervention at 24 hours in the NZW rabbit and the time dependence of intervention with raxibacumab at a dose level of 20 mg/kg administered at either 24 or 36 hours post inhalation exposure to anthrax spores.

This study was a further exploration of therapeutic treatment in the rabbit. The results of Study 358-N005999 showed partial protection against inhalation anthrax mortality with raxibacumab treatment at 24 hours post challenge, as compared with 100% protection at

earlier time points and this time point was chosen for examination of dose ranging. The difference in the effect of an intermediate dose (20 mg/kg) was also examined at 24 and 36 hours.

Study Design and Analysis

This study comprised 72 naïve rabbits randomly divided into 6 groups of 12 animals (6 males and 6 females). The rabbits assigned to the control group received raxibacumab diluent by IV injection at the 24 hour time point. The animals in Groups 2-5 received a single IV injection of raxibacumab (5, 10, 20, or 40 mg/kg) at 24 hour after anthrax spore challenge. The animals in Group 6 received a single IV injection of raxibacumab (20 mg/kg) at 36 hours after anthrax spore challenge. The target challenge dose was 100 x LD_{50} (Ames strain).

This study design had 80% power at a 5% significance level to detect an absolute improvement of 58.4% or more in the 14-day survival in any 1 of the raxibacumab arms. An 8.3% (1 of 12) survival at Day 14 in the vehicle control group and a 66.7% (8 of 12) survival at Day 14 in any of the raxibacumab treatment groups were assumed. Power calculations were performed assuming 2-sided Fisher's exact test for the pairwise comparisons of lethality rates between the vehicle control group and 1 of the raxibacumab treatment groups.

The primary efficacy endpoint for the study was survival at Day 14. The primary analysis of the study was to compare the difference in the survival at Day 14 between any 1 of the raxibacumab treatment groups and the vehicle control group using a 2-tailed Fisher’s exact test.

The secondary endpoint for the study was survival time, defined as the time from spore challenge to death during the 14-day study. The survival time of the rabbits that survive at the end of the follow-up was censored at the 14-day study period. The survival time between any 1 of the raxibacumab treatment groups and the vehicle control group was compared using Kaplan-Meier analysis.

The statistical analysis was performed in accordance with the prespecified analytical plan. The primary analysis was performed on the ITT population. All deaths were defined as failures in the primary analysis. All statistical tests assume a significance level of 0.05, unless otherwise specified.

In addition to survival rates and time, this study included collection of clinical observations.

Results

The mean challenge dose among treatment groups ranged from 97.3 to 157.9 x LD_{50} (mean for all groups 128.1 x LD_{50}) and there was no significant difference in the inhaled dose among the 6 groups ($p = 0.3380$), although the 20 mg/kg 24-hour raxibacumab treatment group had the lowest mean spore challenge.

The primary efficacy analysis of the study compared the difference in the survival at Day 14 between each of the raxibacumab treatment groups and the vehicle control group. There was a significant difference in the 14-day survival among all 6 groups in the study ($p = 0.0231$, Table 13-29 and Figure 13-37). The 14-day survival was significantly higher in the 24-hour 20 mg/kg group (42%) compared with the vehicle control group (0%), $p = 0.0373$.

Table 13-29 Survival at Day 14 (371-N006101)

Treatment	Number (%) of Survivors	P-value vs Control[1]
24h-Diluent	0 (0%)	-
24h-5 mg/kg raxibacumab	3 (25%)	0.2174
24h-10 mg/kg raxibacumab	4 (33.3%)	0.0932
24h-20 mg/kg raxibacumab	5 (41.7%)	0.0373
24h-40 mg/kg raxibacumab	4 (33.3%)	0.0932
36h-20 mg/kg raxibacumab	0 (0%)	1.0000

[1] From a 2-sided Fisher's exact test for the comparison vs the 24 hour diluent group (p-value for the comparison among all groups is 0.0231).

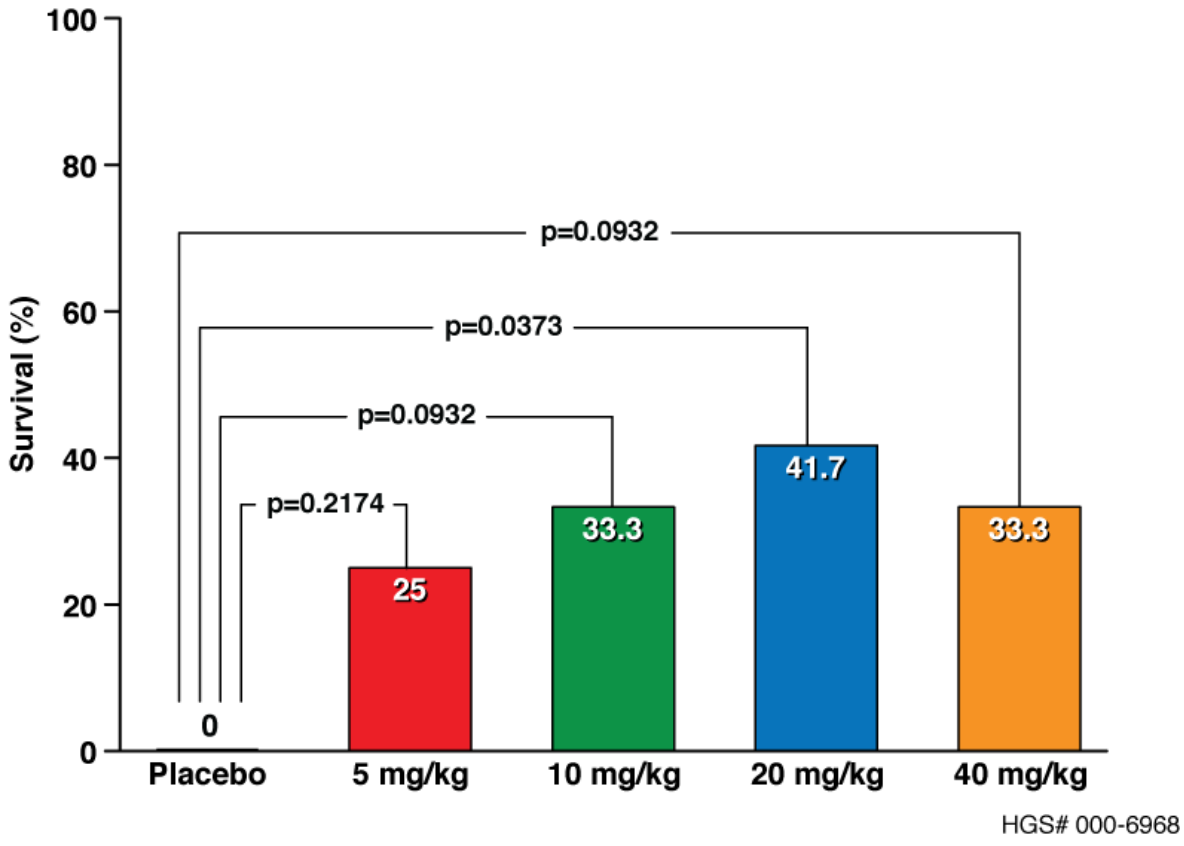

Figure 13-37 Survival at Day 14 (371-N006101)

The Cochran-Armitage test was used to examine the dose response trend of the survival at Day 14 in those rabbits administered raxibacumab at 24 hours after spore challenge. The survival time was significantly higher in the 24-hour 20 mg/kg group compared with vehicle control group, p = 0.0020 (Table 13-30). In the group of rabbits that were treated with 20 mg/kg raxibacumab at 24-hours after spore challenge, the median survival time was 6 days compared with 3 days in the vehicle control group.

There was a significant dose-response trend of survival at Day 14 (p = 0.0390) among the 24-hour dosing groups (Figure 13-38). There was also a significant time-response trend with respect to survival at Day 14 (p = 0.0141) among the 20 mg/kg dosing groups, assuming the rabbits in the diluent control group would receive raxibacumab at an essentially infinitely late time.

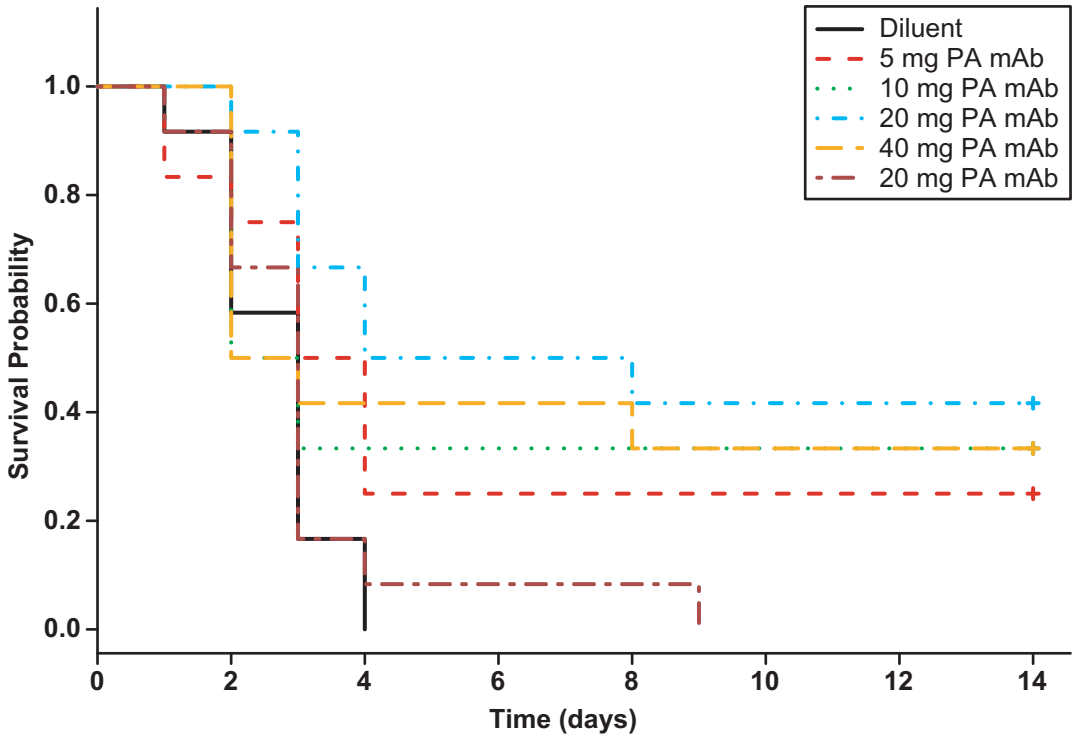

Figure 13-38 Survival curves (371-N006101)

Diluent or 5 to 40 mg/kg raxibacumab was given IV 24 hours after anthrax spore challenge. In a separate treatment group, 20 mg/kg raxibacumab (2nd listing in figure legend) was given IV 36 hours after anthrax spore challenge.

Table 13-30 Survival time analysis (371-N006101)

Treatment	Median Survival Time (days)	P-values vs Control[1]
24h-Diluent	3.0	-
24h-5 mg/kg raxibacumab	3.5	0.0770
24h-10 mg/kg raxibacumab	2.5	0.2408
24h-20 mg/kg raxibacumab	6.0	0.0020
24h-40 mg/kg raxibacumab	2.5	0.0888
36h-20 mg/kg raxibacumab	3.0	0.6019

[1] From a log-rank test for the comparison vs the 24 hour diluent group (p-value for the comparison among all groups is 0.0735).

At the time this study was performed, serum PA was not being measured nor was testing for bacteremia included in this study. Consequently, the disease status of the animals at the time of treatment is uncertain. In retrospect, based on the results of the rabbit characterization study (Study 615-N104504) and the pivotal rabbit efficacy study (Study 682-G005758), the onset of systemic disease occurs generally in the time frame between 20 and 40 hours following anthrax spore challenge in rabbits. The interpretation of this study is therefore complicated by the fact that the animals in the 24 and 36 hour treatment groups were likely a mixed group of symptomatic and asymptomatic animals, and hence had a range of risk of mortality.

Conclusions

- The 14-day survival benefit was raxibacumab dose-dependent among the 24-hour dosing groups.

13.12.4 Pre-exposure Prophylaxis in the Monkey (Study 290-N005433)

This GLP study, "Evaluation of the Prophylactic Efficacy of HGSI PA Monoclonal Antibody Against Aerosolized Anthrax in the Cynomolgus Monkey" (290-N005433) evaluated the efficacy of raxibacumab in protection against lethality when given as a prophylactic agent.

Study Design and Analysis

This study comprised 40 naïve monkeys divided into 4 groups of 10 animals per group (5 males and 5 females). The control group received raxibacumab diluent by SC injection 48 hours prior to aerosol challenge. Monkeys in the active treatment groups received a SC injection of raxibacumab at dose levels of 10, 20, and 40 mg/kg 48 hours prior to aerosol challenge. On Study Day 0, all monkeys were exposed to *B. anthracis* spores (Ames strain) at a target dose of 100 x LD_{50}. The SC injection was administered 2 days before spore challenge so that raxibacumab would reach peak concentrations at the time of spore challenge.

The sample size for this study was determined based on the primary efficacy endpoint of survival at Day 28. This study design had 84% power at the 5% significance level to detect an absolute improvement of 70% or more in the 28-day survival in any 1 of the raxibacumab

treatment arms, based on the assumption of 10% (1 of 10) survival at Day 28 in the vehicle control.

The statistical analysis was performed in accordance with the prespecified analytical plan. The primary analysis was performed on the randomized and treated population. All deaths were defined as failures in the primary analysis.

The primary endpoint analysis was the Fisher's exact test for the 28-day survival analysis. As a secondary analysis, Kaplan-Meier methods were used to analyze the monkey's survival time from spore challenge to death during the 28-day study period. All statistical tests were 2-sided and performed at a significance level of 0.05, unless otherwise specified.

In addition to survival rates and time, this study included evaluation of bacteremia and raxibacumab PK.

Results

The mean challenge dose among treatment groups ranged from 180.0 to 194.4 x LD_{50} and there was no significant difference in the inhaled dose among the 6 groups (p = 0.9571).

The primary efficacy analysis of the study compared the difference in the survival at Study Day 28 between any 1of the raxibacumab treatment groups and the control group. There was a significant difference in the 28-day survival between the vehicle control and raxibacumab treatment groups ($p < 0.0001$, Table 13-31 and Figure 13-39). The 28-day survival was significantly higher in the 10 mg/kg (60%, p = 0.108), 20 mg/kg (70%, p = 0.0031), and 40 mg/kg SC groups (90%), p = 0.0001, then in the vehicle control group.

Table 13-31 Survival at Day 28 (290-N005433)

Treatment	Number (%) of Survivors	P-value vs Control[1]
Vehicle	0 (0%)	-
10 mg/kg raxibacumab SC	6 (60%)	0.0108
20 mg/kg raxibacumab SC	7 (70%)	0.0031
40 mg/kg raxibacumab SC	9 (90%)	0.0001

[1] From a 2-sided Fisher's exact test. The p-value for the comparison among all groups is < 0.0001.

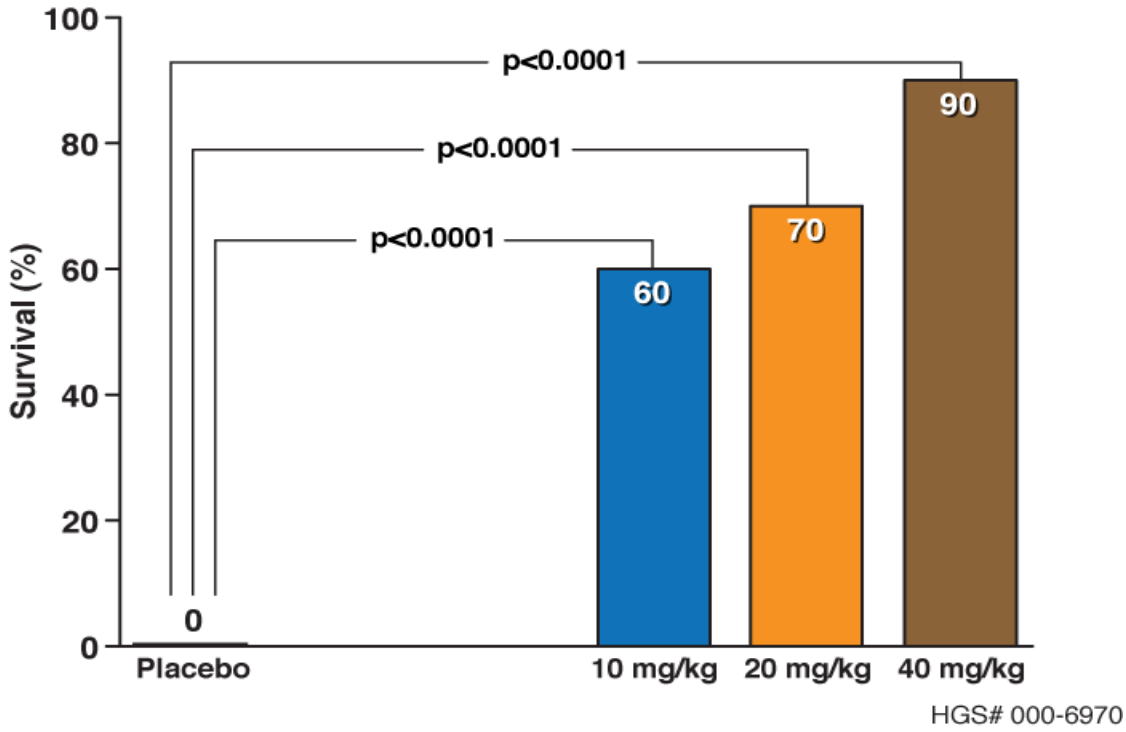

Figure 13-39 Survival at Day 28 (290-N005433)

The survival time between any 1 of the raxibacumab treatment groups and the vehicle control group was compared using the Kaplan-Meier method. The survival time of the monkeys was significantly different between the vehicle control and raxibacumab treatment groups ($p < 0.0001$, Figure 13-40). The median survival time was significantly longer among all the active treatment groups compared to the vehicle control group, all $p \leq 0.0005$. The median survival time was 4 days for the vehicle control group compared with more than 28 days for the raxibacumab treatment groups. The Cochran-Armitage test was used to examine the dose response trend of survival at Day 28. There was a significant dose-response trend with respect to survival at Day 28 ($p = 0.0002$). There was no significant difference in the 28-day survival or in the time to death between male and female monkeys.

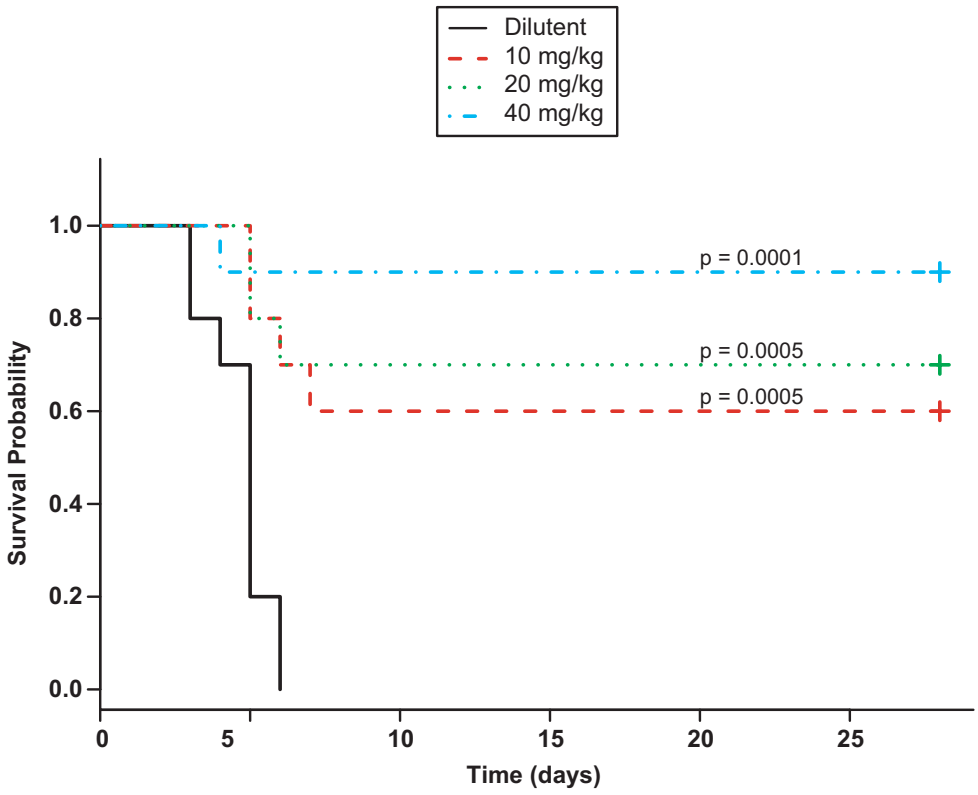

Figure 13-40 Survival curves (290-N005433)

All p-values were obtained from a log-rank test. The p-values for the comparison among all groups are < 0.0001. The p-values marked in the graph are for the comparison vs the vehicle.

With respect to bacteremia, no bacteremia was detected in any of the 22 surviving raxibacumab treatment monkeys at Days 7, 14, 21, and 28. Incidence of bacteremia during the study was significantly lower in all animals in the raxibacumab treatment groups compared with vehicle control ($p < 0.02$). During the study, terminal bacteremia was detected in 9 of the 10 monkeys in the vehicle control group, while in all 3 raxibacumab treatment groups; terminal bacteremia was detected in only 6 monkeys during the study (3 in the 10 mg/kg group, 2 in the 20 mg/kg group, and 1 in the 40 mg/kg group).

A complete necropsy examination was performed on all monkeys that died or were euthanized for humane reasons. Lesions present in all monkeys that succumbed to challenge were typical of inhalation anthrax. The most common anthrax-related lesions in this study were splenic enlargement, enlargement and/or discoloration (hemorrhage) of lymph nodes (especially bronchial), and edema and/or hemorrhages in various organs. There was no apparent biologically significant difference in the pattern of lesions between treatment groups.

Conclusions

- Raxibacumab provided statistically significant protection against mortality compared with control and the effect was dose-dependent.
- Raxibacumab prolonged survival time. There was no significant difference in clinical protection between male and female animals.
- No bacteremia was detected in any of the 22 surviving monkeys.

13.12.5 Long-term Protective Efficacy of Raxibacumab the Monkey (Study 374-N006090)

This non-GLP study, "Evaluation of the Long Term Protective Efficacy of PA mAb in the Cynomolgus Monkey" (374-N006090) evaluated if monkeys previously protected against lethality by the prophylactic administration of raxibacumab were able to mount an immune response to anthrax infection such that they could survive a spore rechallenge approximately 11 months after the 1st challenge.

Study Design and Analysis

This study comprised 21 monkeys (11 male and 10 female) that had survived the anthrax spore challenge in Study 290-N005433 and 6 naïve (3 males and 3 female) monkeys that served as a control group. On Study Day 0, all monkeys were exposed to *B. anthracis* spores (Ames strain) at a target dose of 100 x LD_{50}. None of the monkeys received any treatment during this study.

The primary endpoint of the study was Day 28 survival. Blood was collected from monkeys for analysis of serum anti-PA levels and neutralizing anti-PA levels prior to aerosol challenge and on Days 14 and 28. Bacteremia was measured on terminal blood samples to confirm anthrax infection.

Results

The mean challenge dose in the control group was 154 x LD_{50} and 179 x LD_{50} in the raxibacumab treatment group and there was no significant difference in the inhaled dose between the groups.

All of the monkeys (n = 21) previously treated with raxibacumab survived the anthrax spore rechallenge in contrast to the control monkeys (n = 6) all of which died within 2 to 6 days. Both the survival rates and the median time to death (3 days in the control group and > 28 days in the rechallenged group), were statistically significantly different between the control and rechallenged groups ($p < 0.0001$, for survival rate and time to death (TTD). All monkeys in the control group were bacteremic at death and gross necropsy examinations confirmed lesions typical of inhalation anthrax.

Sixteen out of the 22 monkeys that survived the 1st spore challenge (Study 290-N005433) generated a statistically significant PA neutralizing antibody response (as detected by the

cAMP-induction bioassay) and all 22 monkeys exhibited an increase in total anti-PA titers ranging from a 5 to 74-fold increase over baseline (average of 28 ± 22).

In the 21 monkeys enrolled in this study, following the 2nd spore challenge (approximately 11 months following initial spore challenge), in the absence of raxibacumab treatment, all previously-treated monkeys mounted a protective immune response. Neutralizing antibodies were detected in all animals with an average 56-fold increase compared with levels following the initial exposure and total anti-PA titers increased from an average of 28-fold after 1st exposure to 483-fold following the re-challenge.

Animals dying on study had a gross necropsy performed on all major body tissues. Lesions present in these monkeys were typical of inhalation anthrax.

Conclusions

- Monkeys that were treated with raxibacumab and survived anthrax spore challenge mounted an immune response that completely protected them from re-challenge with anthrax spores approximately 1 year later.
- All control animals succumbed to anthrax disease following spore challenge.
- Raxibacumab treatment does not interfere with the ability of the monkeys to mount an immune response to PA.

13.13 Appendix 13: Histology in Anthrax-treated Animals

Microscopic examination of tissues from animals that died or were euthanized on study was performed to confirm the presence of anthrax disease (Table 13-32). The tissues involved and the observations made are qualitatively the same as reported in the literature for rabbits (Zaucha et al, 1998) and cynomolgus monkeys and other non-human primates succumbing to inhalation anthrax, including rhesus and African Green monkeys (Vasconcelos et al, 2003; Friedlander et al, 1993; Fritz et al, 1995; and Twenhafel et al, 2007). In the monotherapy studies, among the non-survivors, the incidence and severity of histopathology findings was generally the same or greater in the placebo treatment group compared with the raxibacumab treatment groups for all tissues, except brain.

With respect to the findings in the brain, the incidence and severity of brain findings was not greater in the 40 mg/kg raxibacumab treatment group than in the 20 mg/kg raxibacumab treatment group. Moreover, raxibacumab did not cause more rapid death among non-survivors and the median time to death was numerically greater in the raxibacumab treatment groups compared with the placebo groups: rabbits, 2.7 days for the placebo treatment group, 3.0 days for the 20 mg/kg raxibacumab treatment group and 2.9 days for the 40 mg/kg raxibacumab treatment group; monkeys, 3.3 days for the placebo treatment group, 3.9 days for the 20 mg/kg raxibacumab treatment group and 3.7 days for the 40 mg/kg raxibacumab treatment group. These findings suggest that raxibacumab treatment did not exacerbate the progression of disease among non-survivors.

In Study 781-G923701, in which the surviving rabbits in the active treatment groups were sacrificed at the end of the study (all of the placebo-treated rabbits died on study), none of the rabbits had evidence of brain lesions. This finding suggests that raxibacumab administration to animals with systemic anthrax disease in the presence of active antimicrobial therapy does not increase the occurrence of brain lesions. Moreover, the clinical observations of surviving animals in the monkey studies (724-G005829 and 789-G923702) do not indicate any emerging or continuing disability due to CNS involvement.

Taken together, the effects of raxibacumab on survival rate and time and pathophysiology are consistent across the rabbit and monkeys efficacy studies. Raxibacumab significantly increases survival and survival time compared with placebo. Among the non-survivors, raxibacumab treatment is associated with greater protection of highly-vascularized organs suggesting that bacteria and anthrax toxin are cleared from these organs, reducing the incidence of sepsis and death. In the animals that have brain involvement, although raxibacumab treatment is associated with a higher incidence and severity of brain lesions, these animals do not die more rapidly than animals without brain findings. This is consistent with bacteria in the brain being cleared less well than in highly-vascularized tissues and thus causing increased inflammation and hemorrhage. Importantly, surviving animals, all of which received raxibacumab, did not have any evidence of CNS involvement while on study, at the end of the study or in subsequent follow up. Raxibacumab may not prevent meningeal pathology if bacteria become established in the brain, but raxibacumab does reduce widespread sepsis and overall mortality.

Table 13-32 Histology findings across studies

Incidence (Severity[1]) – Non-survivors												
	Placebo				Raxibacumab				Antibiotic		Antibiotic + Raxibacumab	
					20 mg/kg	40 mg/kg	20 mg/kg	40 mg/kg	Levo	Cipro	Levo/ Raxi	Cipro/ Raxi
Tissue/Observation	682-G005758 (n = 16)	724-G005829 (n = 12)	781-G923701 (n = 12)	789-G923702 (n = 12)	682-G005758 (n = 12)	682-G005758 (n = 11)	724-G005829 (n = 7)	724-G005829 (n = 5)	781-G923701 (n = 1)	789-G923702 (n = 0)[2]	781-G923701 (n = 1)	789-G923702 (n = 2)
Lymph node, bronchial		(n = 8)					(n = 3)	(n = 3)				
Bacteria	15 (3.0)	8 (1.3)	12 (3.1)	5 (1.3)	3 (0.8)	5 (1.2)	2 (0.7)	1 (0.5)	0	-	0	1 (1.5)
Hemorrhage	12 (1.8)	5 (1.5)	12 (2.7)	5 (2.2)	10 (2.0)	6 (1.6)	1 (0.3)	1 (0.5)	0	-	0	1 (0.5)
Inflammation	15 (3.0)	1 (0.3)	9 (1.6)	-	12 (3.2)	11 (2.5)	1 (0.3)	0	1 (1.0)	-	1 (3.0)	1 (1.5)
Necrosis	16 (3.4)	8 (2.1)	12 (3.4)	6 (3.0)	12 (2.9)	9 (2.5)	3 (1.0)	0	1 (1.0)	-	0	0
Lymph node, mediastinal		(n = 11)										
Bacteria	13 (3.1)	11 (1.4)	12 (3.2)	12 (1.7)	3 (0.8)	5 (1.3)	5 (0.9)	2 (0.4)	0	-	0	0
Hemorrhage	14 (1.9)	6 (1.1)	11 (2.5)	6 (1.4)	10 (2.0)	6 (1.8)	1 (0.1)	2 (0.4)	0	-	0	0
Inflammation	12 (2.6)	0	9 (1.6)	-	12 (2.3)	10 (2.6)	0	0	0	-	1 (2.0)	0
Necrosis	15 (3.1)	10 (1.8)	12 (3.4)	10 (2.3)	8 (2.0)	8 (2.5)	4 (0.9)	4 (1.0)	1 (2.0)	-	0	0
Lung												
Bacteria	15 (2.9)	11 (2.2)	12 (2.8)	11 (2.0)	2 (0.2)	1 (0.1)	3 (0.4)	4 (1.2)	1 (2.0)	-	0	0
Hemorrhage	5 (0.6)	3 (0.4)	11 (2.5)	3 (0.5)	1 (0.1)	0	1 (0.1)	0	1 (2.0)	-	1 (2.0)	1 (1.0)
Inflammation	9 (1.0)	6 (0.6)	9 (1.6)	3 (0.3)	7 (0.8)	7 (0.8)	2 (0.3)	1 (0.2)	1 (2.0)	-	1 (1.0)	1 (1.0)
BALT necrosis	1 (0.1)	2 (0.3)	12 (3.4)	1 (0.2)	-	0	-	-	1 (4.0)	-	1 (1.0)	0
Kidney												
Bacteria	15 (2.0)	11 (1.8)	12 (2.6)	12 (1.8)	4 (0.6)	3 (0.6)	3 (0.4)	4 (0.6)	0	-	0	0
Hemorrhage	7 (0.4)	0	1 (0.1)	0	3 (0.5)	2 (0.3)	0	0	0	-	0	0
Inflammation	0	0	0	0	6 (0.8)	1 (0.2)	1 (0.1)	0	0	-	0	0
Necrosis	1 (0.1)	0	4 (0.4)	2 (0.3)	2 (0.3)	3 (0.4)	0	0	0	-	0	0

Table 13-32 Histology findings across studies

Incidence (Severity[1]) – Non-survivors

	Placebo				Raxibacumab				Antibiotic		Antibiotic + Raxibacumab	
					20 mg/kg	40 mg/kg	20 mg/kg	40 mg/kg	Levo	Cipro	Levo/ Raxi	Cipro/ Raxi
Tissue/Observation	682-G005758 (n = 16)	724-G005829 (n = 12)	781-G923701 (n = 12)	789-G923702 (n = 12)	682-G005758 (n = 12)	682-G005758 (n = 11)	724-G005829 (n = 7)	724-G005829 (n = 5)	781-G923701 (n = 1)	789-G923702 (n = 0)[2]	781-G923701 (n = 1)	789-G923702 (n = 2)
Liver												
Bacteria	-	11 (1.76)	12 (1.7)	11 (1.8)	-	-	3 (0.4)	3 (0.6)	0	-	0	0
Hemorrhage	-	-	-	-	-	-	-	-	0	-	0	-
Inflammation	0	-	-	-	1 (0.1)	0	-	-	-	-	-	-
Necrosis	6 (0.8)	2 (0.3)	1 (0.2)	4 (0.5)	5 (0.7)	3 (0.7)	1 (0.1)	0	-	-	-	0
Spleen												
Bacteria	15 (3.1)	10 (3.9)	12 (3.2)	11 (3.3)	2 (0.3)	1 (0.1)	4 (0.9)	3 (1.6)	0	-	0	0
Hemorrhage	-	0	0	-	-	-	0	0	-	-	-	-
Inflammation	7 (0.8)	0	0	4 (0.7)	4 (0.3)	4 (0.5)	0	0	-	-	-	0
Necrosis	8 (1.2)	11 (1.9)	12 (2.6)	12 (2.2)	2 (0.2)	1 (0.3)	5 (0.9)	5 (1.4)	1 (2.0)	-	0	2 (1.0)
Fibrin exudation	13 (2.0)	7 (0.8)	12 (2.2)	10 (0.8)	9 (1.8)	8 (2.1)	3 (0.6)	2 (0.6)	0	-	0	0
Brain												
Bacteria	3 (0.3)	11 (1.2)	8 (0.8)	11 (1.2)	9 (2.2)	7 (1.5)	5 (2.3)	5 (2.0)	0	-	0	1 (1.5)
Hemorrhage	4 (0.5)	2 (0.3)	5 (0.6)	2 (0.4)	10 (1.9)	7 (1.7)	6 (2.6)	4 (2.0)	0	-	0	1 (0.5)
Inflammation	2 (0.2)	1 (0.3)	1 (0.2)	1 (0.2)	8 (1.3)	5 (1.2)	6 (2.6)	3 (1.4)	0	-	0	1 (1.5)
Necrosis	0	1 (0.2)	1 (0.1)	-	5 (0.7)	6 (1.0)	3 (0.7)	2 (0.4)	0	-	0	-

[1] Severity is measured on a scale of 1 to 4, where 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked.

[2] One animal was euthanized outside the index study period up for complications of a hematoma. This animal's lesions were confined to the skin.

(concluded)

Given the pathophysiology of anthrax and the inability of raxibacumab to cross the blood brain barrier, the likely explanation for the increased brain lesions in raxibacumab-treated animals is that raxibacumab is unable to prevent the damaging effects of toxins produced by bacteria that reach the brain. There is no evidence to suggest that raxibacumab causes a general increase in inflammation in the body, as the visceral organs of raxibacumab-treaetd animals tend to have less bacteria, inflammation and hemorrhage than placebo-treaetd controls. Consequently, it is proposed that the increased incidence of brain involvement in anthrax-infected animals that die is not evidence of a safety issue for raxibacumab.

13.14 Appendix 14: Human Safety Studies

13.14.1 PAM-NH-01

This study, PAM-NH-01: A Phase 1, Single-Blind, Placebo-Controlled, Single-Injection, Dose-Escalation Study to Evaluate the Safety and Pharmacokinetics of PA mAb (Human Monoclonal Antibody to *B. anthracis* Protective Antigen) in Healthy Subjects, was conducted at a single clinical site in the US from June 2003 to February 2004.

The objectives of the study were to evaluate the safety and PK of intramuscular (IM) and IV administered raxibacumab in healthy subjects and to define a safe dose for an expanded safety study in healthy subjects.

Study Design and Analysis

This was a Phase 1, single-blind, placebo-controlled, single-injection, dose-escalation study of raxibacumab in healthy subjects. The study was designed to evaluate the safety and PK of 3 single doses of IM administered raxibacumab (0.3, 1, and 3 mg/kg) and up to 5 single doses of IV administered raxibacumab (1, 3, 10, 20, and 40 mg/kg). Two injection sites were evaluated for the IM route of administration: gluteus maximus (all 3 IM dose levels) and vastus lateralis (1 and 3 mg/kg only). The phases of the study included screening (Days -28 to -1; subject eligibility and baseline assessments), insubject (Days 0-3 for IM cohorts and Days 0-2 for IV cohorts; study agent dosing on Day 0 and postdose assessments), outsubject (Days 4-42; follow-up assessments), and exit (Day 56; final assessments).

Raxibacumab was supplied as a liquid formulation and stored in sterile, single-use vials. Each vial contained 50 mg/mL raxibacumab in 10 mM sodium citrate, 1.8% glycine, 1.0% sucrose, and 0.02% (w/v) polysorbate 80, pH 6.5. The product used in this study was manufactured by the M10 manufacturing process. The formulation is the same as that in the product proposed for licensure. The product was supplied in 3 mL and 10 mL vials, with fill volumes of 1.1 and 5.3 mL, respectively, to accommodate the range of doses administered in this study. Placebo was supplied as a liquid formulation and stored in sterile, single-use vials similar to active study agent. Each vial contained 10 mM sodium citrate, 1.8% glycine, 1.0% sucrose, and 0.02% (w/v) polysorbate 80, pH 6.5. Placebo was administered as described for raxibacumab, according to cohort designation.

Raxibacumab was administered via IV and IM routes. The doses for IV administration were 1, 3, 10, 20, and 40 mg/kg, administered as a single dose on Day 0, infused over a minimum period of 2 h. The doses for IM administration were 0.3, 1, and 3 mg/kg into the upper-outer quadrant of the right or left gluteus maximus, and 1 and 3 mg/kg into the anterolateral thigh (vastus lateralis), using a 21-gauge 2-inch needle. If the dosing volume to be injected exceeded 5 mL for the gluteus maximus or 4 mL for the vastus lateralis, the dose was equally divided and injected at 2 sites into the appropriate muscle.

Safety variables included type, frequency, severity, and duration of AEs; changes in clinical laboratory parameters and vital signs; physical examination; and immunogenicity of raxibacumab. The PK parameters of interest were area under the curve (AUC), clearance (CL,

CL/F), volume of distribution (V_z, V_z/F), maximum concentration (C_{max}), time of maximum concentration (t_{max}), terminal elimination half-life ($t_{1/2,\ term}$), and mean residence time (MRT). The pharmacodynamic variable of interest was the in vitro pharmacologic activity of raxibacumab in serum samples, as assessed by cAMP induction.

All statistical tests were 2-sided and performed at a significance level of 0.05 unless otherwise specified. For safety analyses, the modified intent-to-treat (ITT) population was used, defined as the subset of all randomized subjects who received study agent. The modified ITT analysis was based on the planned treatment group rather than the actual treatment received. As planned, the placebo-treated subjects in each mode of administration (IM gluteus maximus, IM vastus lateralis, and IV) were pooled together by injection mode to form 3 corresponding control groups.

For PK analyses, raxibacumab drug concentration-time data were analyzed using noncompartmental methods using WinNonlin Version 4.1. Descriptive statistics were calculated to summarize PK parameters for each cohort. For pharmacodynamics (PD) analyses, raxibacumab bioactivity-time data were analyzed using noncompartmental PK analysis using WinNonlin Version 4.1. Descriptive statistics were calculated to summarize bioassay results and PD parameters for each cohort.

Subject Disposition

In total, 240 subjects were screened to provide 105 eligible subjects for this study. The large number of screening failures was primarily due to individuals not meeting eligibility criteria according to the protocol (88 of the 135 failures), with the most common eligibility failure being screening laboratory values out of the specified range for the study (ie, values had to be Grade 0, or Grade 1 and considered not clinically significant by the investigator). In total, 105 subjects were randomized to and treated in 10 cohorts. Of these 105 subjects, 102 (97%) completed the study. No subjects in this study withdrew due to an AE.

The PK data from the HGS1021-C1064 study are described in Appendix 13.15.1.3.1.

Conclusions

- Raxibacumab is safe and well tolerated when administered as a single dose to healthy subjects at dose levels ranging from 1 to 40 mg/kg IV and from 0.3 to 3 mg/kg IM in the gluteus maximus or vastus lateralis muscle.
- The mean elimination half-life of raxibacumab is 16 to 19 days following a single IV administration and 15 to 19 days following a single IM administration. The t_{max} is approximately 6 days for an IM dose.
- Absolute bioavailability was 71% to 85% for the vastus lateralis injection site and 50% to 54% for the gluteus maximus injection site.
- Serum levels of biologically active raxibacumab correlate with the PK profiles of raxibacumab for IV, IM gluteus maximus, and IM vastus lateralis injections.

- None of the 78 healthy adult subjects who were treated with raxibacumab and were evaluated for immunogenicity developed an anti-raxibacumab antibody response.

13.14.2 HGS1021-C1064

This Phase 2/3 study, HGS1021-C1064: "An Open-Label Study to Evaluate the PK and Safety of Raxibacumab (Human Monoclonal Antibody to *B. anthracis* Protective Antigen) Administered in Combination with Ciprofloxacin in Healthy Subjects", was conducted at 3 clinical sites in the US from January to August 2007.

The primary objectives of the study were to determine the effect of co-administration of raxibacumab on orally (PO) administered ciprofloxacin PK and to evaluate the safety of raxibacumab alone and in combination with PO and/or IV administered ciprofloxacin. A secondary objective was to characterize the effect of co-administration of PO and IV ciprofloxacin on raxibacumab PK.

Study Design and Analysis

This was an open-label study to evaluate the safety and PK of combined administration of raxibacumab and ciprofloxacin in healthy adult male and female subjects. Three treatment groups were evaluated (Figure 13-41). One group received a single raxibacumab (40 mg/kg) dose IV on Day 0. One group received PO ciprofloxacin (500 mg Q12h, Days 0 to 7; total of 15 doses), with a single raxibacumab (40 mg/kg) dose IV on Day 5. The third group received a single IV ciprofloxacin (400 mg) dose on Day 0 immediately followed by a single IV raxibacumab (40 mg/kg) dose, a 2nd IV ciprofloxacin (400 mg) dose 12 h later, and then PO ciprofloxacin (500 mg Q12h, Days 1 to 7) for a total of 13 doses. The study phases included screening (subject eligibility and baseline assessments, Days -28 to -1), insubject (study agent dosing and pre- and post-dose assessments, Days 1 to 7), and outsubject (follow-up) assessments to exit (final assessments, 56 days after raxibacumab administration.

Raxibacumab was supplied as a liquid formulation and stored in sterile, single-use vials. Each vial contained 50 mg/mL raxibacumab in 10 mM sodium citrate, 1.8% glycine, 1.0% sucrose, and 0.02% (w/v) polysorbate 80, pH 6.5. The product used in this study was manufactured by the M11 manufacturing process, the process proposed for licensure. Ciprofloxacin was supplied as tablets (500 mg, Teva Pharmaceutical Industries Ltd., Israel) and as a 2 mg/mL stock solution in 5% dextrose (Bayer Pharmaceuticals, West Haven, CT). IV ciprofloxacin was to be administered over 60 minutes at a constant rate. Lot 2500LXV was used for IV ciprofloxacin.

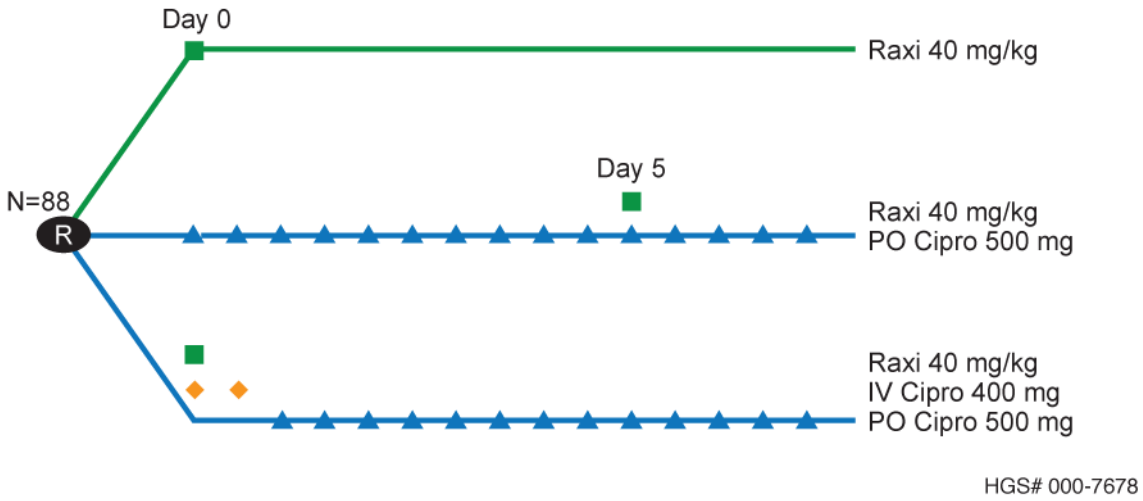

Figure 13-41 Study design for HGS1021-C1064

Raxibacumab, green squares; oral ciprofloxacin, blue triangles; IV ciprofloxacin, yellow diamonds

Safety evaluation included analysis of AEs, clinical laboratory parameters, immune markers (special labs), immunogenicity, physical examinations, and vital signs.

For ciprofloxacin, PK parameters were to be calculated from plasma ciprofloxacin concentration-time results using noncompartmental techniques. For raxibacumab, PK parameters were to be calculated from serum raxibacumab concentration-time results using noncompartmental techniques.

Subject Disposition

In total, 88 subjects were randomized and treated (28 in the raxibacumab only group, 30 in the raxibacumab/POciprofloxacin group and 28 in the raxibacumab/IV+PO ciprofloxacin group); 70 (79.5%) subjects completed the study and 18 (20.5%) subjects withdrew from the study. None of these 18 subjects withdrew due to an AE. Two subjects in Group 1 received ciprofloxacin and withdrew due to subject request prior to receiving raxibacumab.

A requirement to premedicate with oral diphenhydramine was added to the protocol after 6 of the first 25 subjects developed infusion-related skin reactions. Although the raxibacumab-related rashes were mild to moderate in severity, transient, and half resolved without medication, the balance of the subjects were mandated to receive premedication with oral diphenhydramine.

The PK data from the HGS1021-C1064 study are described in Appendix 13.15.3.4.

Conclusions

- Raxibacumab was safe and well tolerated when administered at a dose of 40 mg/kg IV alone or in combination with 500 mg PO or 400 mg IV ciprofloxacin or both.

- Six subjects (6.8%) experienced infusion-related rashes considered related to raxibacumab during the study. The vast majority of subjects (98.4%, 60 of 61) who were premedicated with PO diphenhydramine did not develop a raxibacumab-related infusion reaction.
- There were no constitutional symptoms evidenced by vital sign changes or abnormal laboratory values in the subjects with rash that would indicate a systemic hypersensitivity or anaphylactoid reaction beyond the localized cutaneous reactions.
- No subjects treated with raxibacumab in this study had an anti-raxibacumab antibody response.
- Co-administration with IV raxibacumab had no effect on ciprofloxacin PK for PO ciprofloxacin doses.
- Exposure to ciprofloxacin appeared to have no consistent or meaningful impact on raxibacumab PK.
- Raxibacumab exposure was not altered in subjects premedicated with oral diphenhydramine, relative to those who were not, independent of whether or not the subjects experienced rash.

13.14.3 HGS1021-C1069

This Phase 2/3 study, HGS1021-C1069: An Open-Label Study to Evaluate the Immunogenicity and Safety of Raxibacumab (Human Monoclonal Antibody to *B. anthracis* Protective Antigen) Administered in Healthy Subjects was conducted at 2 clinical sites in the US from January to May 2008.

The primary objective of the study was to evaluate immunogenicity in subjects receiving 2 doses of raxibacumab with a delay (ie, washout) prior to the 2nd dose. The secondary objectives of the study were to evaluate safety in subjects receiving 2 doses of raxibacumab with a delay prior to the 2nd dose and to determine serum raxibacumab PK in subjects receiving 2 doses of raxibacumab with a delay prior to the 2nd dose. All of the subjects enrolled in this study had previously received a single administration of raxibacumab in the HGS1021-C1064 study.

Study Design and Analysis

This was an open-label study to evaluate the immunogenicity and safety of raxibacumab in healthy adult male and female subjects.

A maximum of 25 subjects (to include at least 3 female subjects) who had previously received a dose of raxibacumab ≥ 4 months prior were to be enrolled in this study to ensure at least 15 evaluable subjects received a 2nd dose of raxibacumab equal to that of their previous dose. In total, 23 subjects were screened to provide 20 subjects for the study. All 20 subjects were treated per protocol.

Raxibacumab was supplied as a liquid formulation and stored in sterile, single-use vials. Each vial contained 50 mg/mL raxibacumab in 10 mM sodium citrate, 1.8% glycine, 1.0% sucrose, and 0.02% (w/v) polysorbate 80, pH 6.5. The product used in this study was manufactured by the M11 manufacturing process, the process proposed for licensure. Raxibacumab Lot 71044

was administered to the subjects as a 40 mg/kg IV infusion over approximately 2 h and 15 minutes. All subjects were to be treated with PO 50 mg diphenhydramine up to 60 minutes prior to infusion of raxibacumab.

Safety variables included analysis of immunogenicity, analysis of AEs, clinical laboratory parameters, physical examinations, vital signs, and telemetry.

For PK evaluation, individual subjects' serum raxibacumab concentration-time data were analyzed using noncompartmental techniques to determine PK parameters.

Subject Disposition

In total, 20 subjects were treated and all 20 (100%) subjects completed the study.

The PK data from the HGS1021-C1069 study are described in Appendix 13.15.1.3.3.

Conclusions

- None of the subjects in this study had a positive anti-raxibacumab antibody response.
- There were no subjects with severe AEs, SAEs, or with raxibacumab-related AEs. In addition, there were no subjects who discontinued raxibacumab treatment due to AEs.
- There were no rashes reported in this study.
- There were a small number of transient shifts to Grade 2 or higher clinical laboratory values in this study with no consistent pattern of change, and there were no temporally associated AEs.
- Raxibacumab PK was not meaningfully different for 2 single IV doses administered at least 4 months apart.
- The subgroup of subjects enrolled in this study had PK similar to that for all subjects from the prior study.

13.14.4 HGS1021-C1063

This Phase 3 study, HGS1021-C1063: A Randomized, Single-Blind, Placebo-Controlled Study to Evaluate the Safety and Tolerability of Raxibacumab (Human Monoclonal Antibody to *B. Anthracis* Protective Antigen) in Healthy Subjects was conducted at 6 clinical sites in the US from March to July 2008.

The primary objective of the study was to to evaluate the safety and tolerability of IV 40 mg/kg single dose and double dose (2 doses 14 days apart) raxibacumab. The secondary objective of the study was to determine serum raxibacumab concentrations for use in a population PK analysis.

Study Design and Analysis

This was a randomized, single-blind, placebo-controlled study of raxibacumab in healthy subjects. The study was designed to evaluate the safety and tolerability of IV 40 mg/kg single dose and double dose (2 doses 14 days apart) raxibacumab. In total, 322 subjects were randomized to 1 of 2 raxibacumab groups (40 mg/kg double dose, or 40 mg/kg single dose) or to 1 of 2 matching placebo groups at a ratio of 3:1 (raxibacumab:placebo). Subjects were stratified at randomization by age (< age 65 or ≥ age 65), with a target distribution of approximately 15% of the subjects in the single-dose cohorts ≥ age 65. Subject enrollment targeted a population of approximately 35% female, 8% Hispanic, and 12% non-white. Subjects in the double-dose cohorts received doses of raxibacumab or placebo on Days 0 and 14, while subjects in the single-dose cohorts were administered their dose on Day 0. Specimens for serum raxibacumab concentration measurement were collected at selected time points from all subjects.

Raxibacumab was supplied in 50 mL sterile, single-use vials containing a minimum of 34.0 mL of liquid formulation per vial. Each vial contained 50 mg/mL raxibacumab in 0.12 mg/mL citric acid, 2.8 mg/mL sodium citrate, 10 mg/mL sucrose, 18 mg/mL glycine, 0.2 mg/mL polysorbate 80, pH 6.5. The calculated raxibacumab dose to be administered to the subject was diluted in normal saline to a final volume of 250 mL. The rate of raxibacumab infusion was to be 15 mL/h for the first 20 minutes and then adjusted to 125 mL/h for the remainder of the infusion period. With this schedule, approximately 250 mL should have been infused over the course of 2 h and 15 minutes. Raxibacumab was to be stored at 2-8ºC. The material is stable for up to 8 h at room temperature. Raxibacumab Lots 71044 and 71051 were used in this study.

Placebo was raxibacumab formulation buffer, and was also supplied in 50 mL sterile, single-use vials containing a minimum of 34 mL of liquid formulation per vial. Placebo was to be stored at 2-8°C and is stable for up to 8 h at room temperature. Placebo Lot 71043 was used in this study. Subjects were to be treated with oral diphenhydramine (25-50 mg) up to 60 minutes prior to treatment.

Safety variables included the type, frequency, duration, relationship, and severity of AEs, changes in laboratory parameters, physical examinations, vital signs, and immunogenicity. Statistical analyses were performed on the as-treated population unless otherwise specified. All statistical tests were 2-sided and performed at a significance level of 0.05 unless otherwise specified. The baseline of a variable was defined as the value of the variable measured at Day 0 prior to dosing, unless otherwise specified. The frequency of laboratory abnormalities was tabulated by treatment group. Laboratory values were assessed for significant changes from baseline. Immunogenicity data were summarized in tabular form if there were significant numbers of subjects with antibody formation. A listing was provided to show immunogenicity responses for all subjects by visit.

The study was not prospectively designed to power statistical comparisons of raxibacumab PK between subgroups of subjects. No calculations of PK parameters were performed for this

study; the serum raxibacumab concentration data gathered in this study were used in the population PK analysis.

Subject Disposition

In total, 700 subjects were screened to provide 322 randomized subjects for the study. The number of screening failures was primarily due to subjects not meeting inclusion criteria (70), meeting exclusion criteria (129), subject request (68), and other reasons (121) that included screening outside of the screening window, venipuncture difficulties, abnormal lab assessments, and abnormal vital signs. Of the 322 randomized subjects, 320 were treated (72 in the placebo single-dose group, 8 in the placebo double-dose group, 216 in the raxibacumab single-dose group, and 24 in the raxibacumab double-dose group). One subject randomized to the placebo single-dose group had a positive urine drug screen (UDS) and was not dosed per investigator decision. One subject in the raxibacumab single-dose group refused treatment following an unsuccessful initial attempt at venipuncture for IV dosing.

In total, 304 (95.0%) subjects completed the study and 16 (5.0%) subjects did not complete the study. The most common reason for subject withdrawals among placebo-treated subjects was subject request (3/80, 3.8%). One subject in the placebo double-dose group withdrew due to death (see narrative below). A 2nd subject in the placebo double-dose group withdrew from study agent dosing due to an AE of moderate skin infection (not related, resolved) and received only 1 placebo dose. This subject completed all follow-up visits and is considered a completer in the as-treated population (placebo single-dose group). Additionally, 1 subject in the placebo double-dose group and 1 subject in the raxibacumab double-dose group did not receive their 2nd dose of study agent and were assigned to the single-dose group in the as-treated analysis. There were no withdrawals due to AEs among subjects treated with raxibacumab. Subjects lost to follow-up (6/240, 2.5%) constituted the majority of withdrawals among raxibacumab-treated subjects. Other reasons for withdrawals from the study included subject request (4 subjects) and lack of compliance (1 subject), all in the raxibacumab single-dose group.

The PK data from the HGS1021-C1063 study are described in Appendix 13.15.1.3.2.

Conclusions

- Raxibacumab-treated subjects did not have a higher incidence of AEs, related AEs, SAEs, or severe AEs relative to subjects treated with placebo.
- Incidences of AEs in the majority of system organ class (SOC) categories were not higher among raxibacumab-treated subjects relative to placebo, or were otherwise comparable between raxibacumab and placebo-treated subjects.
- Subjects in the raxibacumab double-dose group had a similar incidence of AEs, related AEs, or SAEs relative to subjects in the raxibacumab single-dose group.
- Incidences of the most common AEs in this study were comparable among raxibacumab and placebo-treated subjects, and administration of the 2nd dose of either agent was not associated with a higher incidence of the most common AEs.

- The incidence of study agent-related AEs were not higher among raxibacumab-treated subjects relative to placebo-treated subjects.
- Repeat dosing with raxibacumab did not result in a higher rate of raxibacumab-related AEs.
- One subject in the placebo double-dose group died from injuries sustained in a motor vehicle accident; the event was considered not to be related to study agent. One subject in the raxibacumab double-dose group had a Grade 3 event of cholecystitis recorded as possibly related to raxibacumab; the AE began 10 days after the subject's 2nd raxibacumab infusion.
- There were no AEs related to vital signs in subjects in the raxibacumab double-dose group.
- Seven of the 320 subjects in this study (2.2%) had a Grade 3 or higher laboratory abnormality; 6/217 (2.8%) from the raxibacumab single-dose group, and 1/74 (1.3%) from the placebo single-dose group. Diphenhydramine pretreatment was well-tolerated. The incidence of rash was similar among raxibacumab-treated subjects (2.5%) and placebo-treated subjects (2.5%). All rashes were mild, 4 were related to study drug (all transient), and 2 were ongoing at the end of the study (all not related).
- No subjects developed an anti-raxibacumab antibody response.
- No subjects in the raxibacumab or placebo double-dose groups had a Grade 3 or higher laboratory abnormality.
- The population of subjects enrolled in this study is representative of the United States population, and is suitable for the planned population PK analyses.
- The serum raxibacumab concentrations observed for this study are in agreement with the serum raxibacumab concentration-time profile in a previous study, and should be adequate to support the planned population PK analyses.
- In essentially all raxibacumab-dosed subjects, serum raxibacumab concentrations remained measurable for up to 56 days post dose.

13.15 Appendix 15: Pharmacokinetics in Rabbits, Monkeys and Humans

Table 13-33 summarizes the studies that comprise the clinical pharmacology program for raxibacumab. The studies include raxibacumab PK in healthy rabbits, monkeys and humans and in anthrax-infected raabtis and monkeys. The kinetics of PA in anthrax-infected rabbits and monkeys are described in Appendix 13.16.

Table 13-33 Description of clinical pharmacology studies

Study ID	Title of Report	Study Objective	Study Design and Type of Control	Test Product (s): Dosage Regimen and Route of Administration	Number (M/F)	Study Duration	Location of Study Report
AB50409.INF.0.016 (PK for Covance Study No. 6962-137)	PK of raxibacumab in normal NZW rabbits following a single IV, SC, or IM injection	Raxibacumab PK	Randomized (non-GLP)	Raxibacumab Single dose 1 or 10 mg/kg, IV, IM, SC	2 M, 2 F (per group)	28 Days	5.3.3.1
AB50409.INF.0.017 (PK for Covance Study No. 6962-136)	PK of raxibacumab in normal cynomolgus monkeys following a single IV, SC, or IM dose	Raxibacumab PK	Randomized (non-GLP)	Raxibacumab Single dose 1 or 10 mg/kg, IV, IM, SC	2 M, 2 F (per group)	42 Days	5.3.3.1
PAM-NH-01.PK	PK analysis of a phase 1 single-blind, placebo-controlled, single-injection, dose escalation study to evaluate the safety and PK of raxibacumab in healthy subjects	Raxibacumab safety and PK	Single-blind, dose-escalation, placebo controlled (GCP)	Raxibacumab or placebo (raxibacumab formulation buffer) single dose 0.3, 1, and 3 mg/kg IM (gluteus maximus) 1 and 3 mg/kg IM (vastus lateralis) 1, 3, 10, 20, and 40 mg/kg IV	80 raxibacumab, 25 placebo	56 Days	5.3.3.1
HGS1021-C1063. PK	PK analysis of a randomized, single-blind, placebo-controlled study to evaluate the safety and tolerability of raxibacumab in healthy subjects	Raxibacumab safety and PK	Randomized, single-blind, placebo-controlled (GCP[1])	Raxibacumab or placebo (raxibacumab formulation buffer) single or double dose 40 mg/kg raxibacumab IV single dose on Day 0 or double dose on Days 0 and 14	156 M, 164 F 240raxibacumab (217 single dose, 23 double dose) and 80 placebo (74 single dose, 6 double dose)	56 Days	5.3.3.1

Table 13-33 Description of clinical pharmacology studies

Study ID	Title of Report	Study Objective	Study Design and Type of Control	Test Product (s): Dosage Regimen and Route of Administration	Number (M/F)	Study Duration	Location of Study Report
HGS1021-C1069. PK	PK analysis of an open-label study to evaluate the immunogenicity and safety of raxibacumab administered in healthy subjects	Raxibacumab safety, immunogenicity, and PK	Open-label (GCP[1])	Raxibacumab single dose 40 mg/kg raxibacumab IV Note: All subjects enrolled had received a single 40 mg/kg raxibacumab IV dose at least 4 months prior to receiving treatment in this study	12 M, 8 F	56 Days	5.3.3.1
AB50409.INF.0.027 (PK for Battelle Study No. 288-HGSIRAB)	PK analysis for evaluation of the pre-and post-exposure prophylactic efficacy of raxibacumab against aerosolized anthrax in the NZW rabbit model	Raxibacumab efficacy and PK	Randomized, placebo-controlled (GLP[1])	Placebo (raxibacumab buffer) SC; raxibacumab 1, 5, 10, or 20 mg/kg SC; raxibacumab 40 mg/kg IV *Treatment was administered 2 days prior to anthrax challenge for all SC groups and immediately following challenge for the IV group.	6 M, 6 F (per group)	14 Days	5.3.3.2
615-N104504	Exploratory study to evaluate markers of disease course of *B. anthracis* in NZW rabbits	Disease progression in rabbits, including PA kinetics	Non-GLP	NA	4 M/4 F	7 days	5.3.5.1
AB50409.INF.0.036 (PK for Battelle Study No. 682-G005758)	Raxibacumab PK and PA kinetics during the evaluation of raxibacumab efficacy as therapeutic treatment against inhalation anthrax in the rabbit model	Raxibacumab efficacy, PK, and PA kinetics	Randomized, placebo-controlled (GLP)	Placebo (raxibacumab buffer) or raxibacumab 20 and 40 mg/kg IV *Treatment was administered based on detection of increased temperature or serum PA by individual animal.	10 M, 7 F (20 mg/kg) 11 M, 8 F (40 mg/kg)	14 Days	5.3.3.2

Table 13-33 Description of clinical pharmacology studies

Study ID	Title of Report	Study Objective	Study Design and Type of Control	Test Product (s): Dosage Regimen and Route of Administration	Number (M/F)	Study Duration	Location of Study Report
AB50409.INF.0.028 (PK for Battelle Study No. 290-N005433)	PK analysis for evaluation of the pre-exposure prophylactic efficacy of raxibacumab against aerosolized anthrax in the monkey model	Raxibacumab efficacy and PK	Randomized, placebo-controlled (GLP[1])	Placebo (raxibacumab buffer), raxibacumab 10, 20, and 40 mg/kg SC *Treatment was administered 2 days prior to anthrax challenge.	5 M, 5 F (per group)	28 Days	5.3.3.2
685-G005762	Model characterization study to evaluate markers of disease course of *B. anthracis* in cynomolgus monkeys	Disease progression in monkeys, including PA kinetics	Non-GLP	NA	7 M/1 F	30 days	5.3.5.1
AB50409.INF.0.040 (PK for Battelle Study No. 724-G005829)	Raxibacumab PK and PA kinetics during the evaluation of raxibacumab efficacy as therapeutic treatment against inhalation anthrax in the monkey model	Raxibacumab efficacy, PK, and PA kinetics	Randomized, placebo-controlled, double-blind (GLP)	Placebo (raxibacumab buffer) or raxibacumab 20 and 40 mg/kg IV *Treatment was administered based on detection of serum PA by individual animal.	6 M, 6 F (placebo) 7 M, 7 F (raxibacumab treatment groups)	28 Days	5.3.3.2
AB50409.INF.0.039.2 (PK for Battelle Study No. 723-G005835)	Levofloxacin PK and PA kinetics during a pilot study evaluation of levofloxacin efficacy as therapeutic treatment against inhalation anthrax in the rabbit model	Levofloxacin efficacy, PK, and PA kinetics	Randomized, placebo-controlled (non-GLP)	Levofloxacin 10, 25, and 50 mg/kg once daily x 3 gastric intubation or untreated control *Treatment was administered based on detection of increased temperature by individual animal.	4 M, 4 F (per group); 3 M control	21 Days	5.3.3.4

Table 13-33 Description of clinical pharmacology studies

Study ID	Title of Report	Study Objective	Study Design and Type of Control	Test Product (s): Dosage Regimen and Route of Administration	Number (M/F)	Study Duration	Location of Study Report
AB50409.INF.0.043 (PK for Battelle Study No. 781-G923701)	Levofloxacin and raxibacumab PK, with PA kinetics, during the evaluation of raxibacumab in combination with levofloxacin for post-exposure treatment in the NZW rabbit inhalational anthrax model	Efficacy, PK, and PA kinetics of raxibacumab and levofloxacin combination	Randomized, placebo-controlled, double blind (GLP)	Placebo (raxibacumab buffer + WFI), raxibacumab buffer + levofloxacin 50 mg/kg, or raxibacumab 40 mg/kg + levofloxacin 50 mg/kg; raxibacumab IV, levofloxacin gastric intubation *Treatment was administered based on detection of increased temperature or serum PA by individual animal.	6 M, 6 F (placebo group) 10 M, 10 F (treatment groups)	28 Days	5.3.3.4
AB50409.INF.0.042 (PK for Battelle Study No. 789-G923702)	Ciprofloxacin and raxibacumab PK, with PA kinetics, during evaluation of the efficacy of raxibacumab in combination with ciprofloxacin for therapeutic treatment in the cynomolgus monkey inhalation anthrax model	Efficacy, PK, and PA kinetics of raxibacumab and ciprofloxacin combination	Randomized, placebo-controlled, double blind (GLP)	Placebo (raxibacumab buffer + WFI); raxibacumab buffer + ciprofloxacin 75 mg q12 h x 6; or raxibacumab 40 mg/kg + ciprofloxacin 75 mg q12h x 6; ciprofloxacin gastric intubation, raxibacumab IV *Treatment was administered based on detection of serum PA by individual animal.	6 M, 6F (placebo) 7 M, 7 F (treatment groups)	28 Days	5.3.3.4

Table 13-33 Description of clinical pharmacology studies

Study ID	Title of Report	Study Objective	Study Design and Type of Control	Test Product (s): Dosage Regimen and Route of Administration	Number (M/F)	Study Duration	Location of Study Report
HGS1021-C1064 Clinical PK	Pharmacokinetic analysis of an open-label study to evaluate the pharmacokinetics and safety of raxibacumab administered in combination with ciprofloxacin in healthy subjects	Safety and PK of raxibacumab and ciprofloxacin combination	Randomized (Groups 1 & 2), open-label (GCP[1])	Raxibacumab single dose and/or ciprofloxacin for 7.5 days 500 mg ciprofloxacin PO q12h x 15 doses on Days 0-7 + 40 mg/kg raxibacumab IV on Day 5 (Group 1) 40 mg/kg raxibacumab IV on Day 0 (Group 2) 400 mg ciprofloxacin IV q12h x 2 doses + 40 mg/kg raxibacumab IV on Day 0 and 500 mg ciprofloxacin PO q12h x 13 doses on Days 1-7	43 M, 45 F (Ie, Group 1; 16 M, 16 F, Group 2 13 M, 15 F, and Group 3, 14 M, 14 F)	56 Days	5.3.3.1

[1] PK evaluations performed by the sponsor were not in full compliance with GLP.

(concluded)

13.15.1 PK in Healthy Rabbits, Monkeys and Humans

13.15.1.1 Rabbits

Study AB50409.INF.0.016 evaluated the PK of raxibacumab following a single dose administered IV, subcutaneously (SC), or intramuscularly (IM) in healthy rabbits. In total, 24 NZW rabbits weighing 2.4 to 2.84 kg were randomly assigned to 6 treatment groups. Each group comprised 4 rabbits (2 male and 2 female) and received either 1 or 10 mg/kg raxibacumab (Lot AB50409-M9) via IV, SC (mid-scapular region), or IM (thigh muscle) administration.

Blood was collected from each animal prior to dosing (predose), at 0.083 (IV groups only), 2, 4, and 8 hours, as well as 1, 2, 3, 4, 6, 10, 14, 21 and 28 days postdose. Drug concentrations in serum samples were determined using a sandwich-type enzyme-linked immunosorbent assay (ELISA) format. The lower limit of quantitation (LLOQ) was 0.055 µg/mL in serum.

PK analyses were conducted by compartmental methods. Inspection of the individual drug concentration-time profiles following IV dosing revealed a profile that was biphasic; hence, a 2-compartment model with 1st order elimination from the central compartment was evaluated. To allow evaluation of the absorption rate following SC or IM dosing, the serum drug concentration-time data were subjected to 1-compartmental model fitting with 1st order absorption and elimination. PK parameters were summarized using descriptive statistics (Table 13-34). Serum drug concentration-time profiles were similar for the SC and IM doses and the terminal elimination phase was similar across all 3 injection routes (Figure 13-42).

Raxibacumab was completely absorbed following SC or IM administration, with a bioavailability of ~100%. The mean C_{max} levels were comparable following SC and IM routes; however, the IM route had slightly faster absorption as indicated by the shorter time of maximum serum concentration (t_{max}). Raxibacumab PK was linear across the 10-fold dose range for all 3 routes of administration evaluated and there were no significant difference observed between genders.

Table 13-34 Raxibacumab PK in healthy NZW rabbits

Dose (mg/kg)/ Route		1 mg/kg IV[1]	10 mg/kg IV	1 mg/kg SC	10 mg/kg SC[2]	1 mg/kg IM[3]	10 mg/kg IM[2]
C_{max} (µg/mL)	Mean	25.7	275.9	10.9	106.9	11.6	102.7
	SD	1.4	18.9	2.2	6.4	2.8	11.4
C_{max}/Dose	Mean	0.026	0.028	0.011	0.011	0.012	0.010
(µg/mL)/(µg/kg)	SD	0.001	0.002	0.002	0.001	0.003	0.001
t_{max} (day)	Mean	0.0	0.0	2.9	3.5	2.2	2.3
	SD	NA	NA	1.0	0.4	0.5	0.7
$AUC_{0-\infty}$	Mean	174	1518	182	1931	230	1490
(µg·day/mL)	SD	60	408	84	357	57	415
$AUC_{0-\infty}$/dose	Mean	0.174	0.152	0.182	0.193	0.230	0.149
(µg·day/mL)/(µg/kg)	SD	0.060	0.041	0.084	0.036	0.057	0.042
$t_{1/2,abs}$ (day)	Mean	NA	NA	0.83	0.97	0.46	0.66
	SD	NA	NA	0.43	0.16	0.14	0.51
$t_{1/2,\alpha}$ (day)	Mean	0.40	0.24	NA	NA	NA	NA
	SD	0.22	0.05	NA	NA	NA	NA
$t_{1/2,\beta}$ (day)	Mean	8.7	6.9	8.7	9.6	12.1	8.4
	SD	4.4	2.7	3.4	1.6	0.7	3.8
MRT (day)	Mean	12.0	9.7	13.8	15.3	18.1	13.0
	SD	6.0	3.7	4.7	2.2	0.9	5.0
CL or CL/F	Mean	6.2	6.9	6.8	5.3	4.5	7.2
(mL/day/kg)	SD	1.8	1.8	4.1	1.0	1.0	2.3
V_1 (mL/kg)	Mean	39.0	36.4	NA	NA	NA	NA
	SD	2.1	2.5	NA	NA	NA	NA
V_{ss} or V_{ss}/F (mL/kg)	Mean	66.9	63.2	71.4	72.3	77.9	77.5
	SD	9.9	15.4	7.0	2.0	14.3	17.0
F (%)	Mean	NA	NA	104.6	127.2	132.4	98.2

[1] One animal from this group was excluded from the analysis because it appeared to be misinjected; the t_{max} was 24 hours when an IV injection would be expected to have t_{max} immediately after injection.

[2] From unpaired T-test, there is significant difference in the t_{max} of 10 mg/kg SC and 10 mg/kg IM groups, but not between 1 mg/kg SC and 1 mg/kg IM groups.

[3] One animal from this group was sacrificed prior to the 4 hour timepoint due to a leg injury considered not related to treatment.

Abbreviations: C_{max}, maximum serum drug concentration; t_{max}, time of maximum serum concentration; $AUC_{0-\infty}$, area under the serum drug concentration-time curve from time 0 to infinite time; $t_{1/2,abs}$, absorption half-life; $t_{1/2,\alpha}$, elimination half-life for the 1st phase; $t_{1/2,\beta}$, elimination half-life for the 2nd (terminal) phase; MRT, mean residence time; CL, clearance; CL/F, apparent clearance after SC dosing; CV coefficient of variation; V_1, volume of distribution for the central compartment; V_{ss}, volume of distribution at steady-state; V_{ss}/F, apparent steady-state volume of distribution after SC or IM dosing; F(%), bioavailability; NA, not applicable.

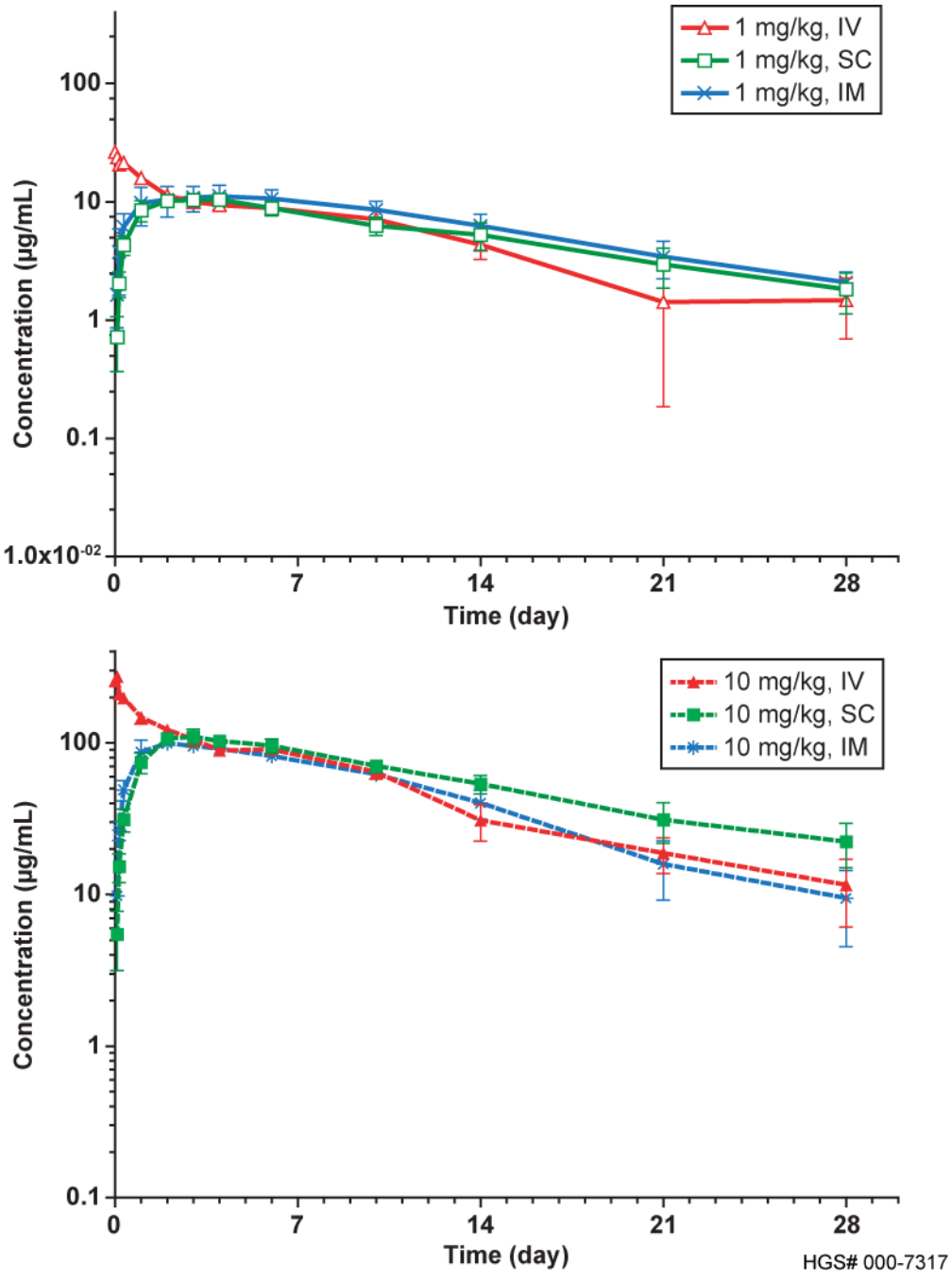

Figure 13-42 **Serum raxibacumab concentrations following a single IV, SC, or IM injection of 1 mg/kg and 10 mg/kg raxibacumab in healthy NZW rabbits (mean ± SD)**

13.15.1.2 Monkeys

Study AB50409.INF.0.017 evaluated the PK of raxibacumab in healthy cynomolgus monkeys following a single IV, SC, or IM injection. In total, 24 cynomolgus monkeys weighing between 2.2 to 3.64 kg were randomly assigned to 6 treatment groups. Each treatment group had 4 monkeys (2 male and 2 female) and received either 1 or 10 mg/kg raxibacumab (Lot AB50409-M9) via IV, SC (mid-scapular region), or IM (thigh muscle) administration.

Blood was collected from each animal prior to dosing (predose), at 0.083 (IV groups only), 2, and 8 hours, as well as 1, 2, 3, 4, 7, 14, 21, 28, 35, and 42 days postdose. Drug concentrations in serum samples were determined using a sandwich-type ELISA format. The LLOQ in monkey serum was 0.0125 µg/mL.

PK analyses were conducted by compartmental methods. Inspection of the individual drug concentration-time profiles following IV dosing revealed a profile that was biphasic; hence, a 2-compartment model with 1st order elimination from the central compartment was evaluated. To allow evaluation of the absorption rate following SC or IM dosing, the serum drug concentration-time data were subjected to a one compartment model with 1st order absorption and elimination. PK parameters were summarized using descriptive statistics (Table 13-35). Figure 13-43 shows serum drug concentration-time profiles for 3 injection routes.

The bioavailability for the IM route (82-115.6%) is slightly higher than that for the SC route (74.9-84.3%), while the C_{max} levels are comparable following SC and IM administration. As was observed in rabbits, the IM route appeared to have a shorter time of maximum serum concentration (t_{max}) than that of the SC route for both dose groups). Overall, raxibacumab PK were linear across the 10-fold dose range evaluated for all 3 injection routes. There were no significant differences between male and female monkeys.

Table 13-35 PK parameters by compartmental analysis in healthy cynomolgus monkeys

Dose (mg/kg)/ Route		1 mg/kg IV	10 mg/kg IV	1[1] mg/kg SC	10[2] mg/kg SC	1[1] mg/kg IM	10[2] mg/kg IM
C_{max} (µg/mL)	Mean	28.8	261.6	10.1	85.2	11.2	114.0
	SD	6.4	30.3	1.6	17.4	1.0	6.1
C_{max}/Dose	Mean	0.029	0.026	0.010	0.009	0.011	0.011
(µg/mL)/(µg/kg)	SD	0.006	0.003	0.002	0.002	0.001	0.001
t_{max} (day)	Mean	0.0	0.0	2.1	2.6	1.1	1.0
	SD	NA	NA	0.7	0.4	0.7	0.2
$AUC_{0-\infty}$ (µg·day/mL)	Mean	267	2030	200	1712	219	2346
	SD	91	172	64	362	29	431
$AUC_{0-\infty}$/dose	Mean	0.267	0.203	0.200	0.171	0.219	0.235
(µg·day/mL)/(µg/kg)	SD	0.091	0.017	0.064	0.036	0.029	0.043
$t_{1/2,abs}$ (day)	Mean	NA	NA	0.45	0.58	0.18	0.16
	SD	NA	NA	0.22	0.15	0.14	0.04

Table 13-35 PK parameters by compartmental analysis in healthy cynomolgus monkeys

Dose (mg/kg)/ Route		1 mg/kg IV	10 mg/kg IV	1[1] mg/kg SC	10[2] mg/kg SC	1[1] mg/kg IM	10[2] mg/kg IM
$t_{1/2,\alpha}$ (day)	Mean	1.10	0.69	NA	NA	NA	NA
	SD	0.84	0.53	NA	NA	NA	NA
$t_{1/2,\beta}$ (day)	Mean	15.8	11.8	12.0	11.9	12.7	13.5
	SD	4.1	1.9	3.6	0.9	0.7	2.2
MRT (day)	Mean	19.8	15.8	18.0	18.1	18.6	19.7
	SD	4.3	1.7	5.0	1.2	1.0	3.1
CL or CL/F (mL/day/kg)	Mean	4.1	5.0	5.4	6.0	4.6	4.4
	SD	1.4	0.4	1.6	1.3	0.6	0.9
V_1 (mL/kg)	Mean	36.0	38.6	NA	NA	NA	NA
	SD	7.8	4.3	NA	NA	NA	NA
V_{ss} or V_{ss}/F (mL/kg)	Mean	78.8	78.0	88.4	103.4	84.5	83.3
	SD	23.7	8.3	15.1	18.4	9.5	3.9
F (%)	Mean	NA	NA	74.9	84.3	82.1	115.6

[1] From unpaired t-test, there is no significant difference between the t_{max} of 1 mg/kg SC and IM dose groups.

[2] From unpaired t-test, there is significant difference between the t_{max} of 10 mg/kg SC and IM groups.

Abbreviations: C_{max}, maximum serum drug concentration; t_{max}, time of maximum serum concentration; $AUC_{0-\infty}$, area under the serum drug concentration-time curve from time 0 to infinite time; $t_{1/2,abs}$, absorption half-life; $t_{1/2,\alpha}$, elimination half-life for the 1st phase; $t_{1/2,\beta}$, elimination half-life for the 2nd (terminal) phase; MRT, mean residence time; CL, clearance; CL/F, apparent clearance after SC dosing; CV coefficient of variation; V_1, volume of distribution for the central compartment; V_{ss}, volume of distribution at steady-state; V_{ss}/F, apparent steady-state volume of distribution after SC or IM dosing; F(%), bioavailability; NA, not applicable.

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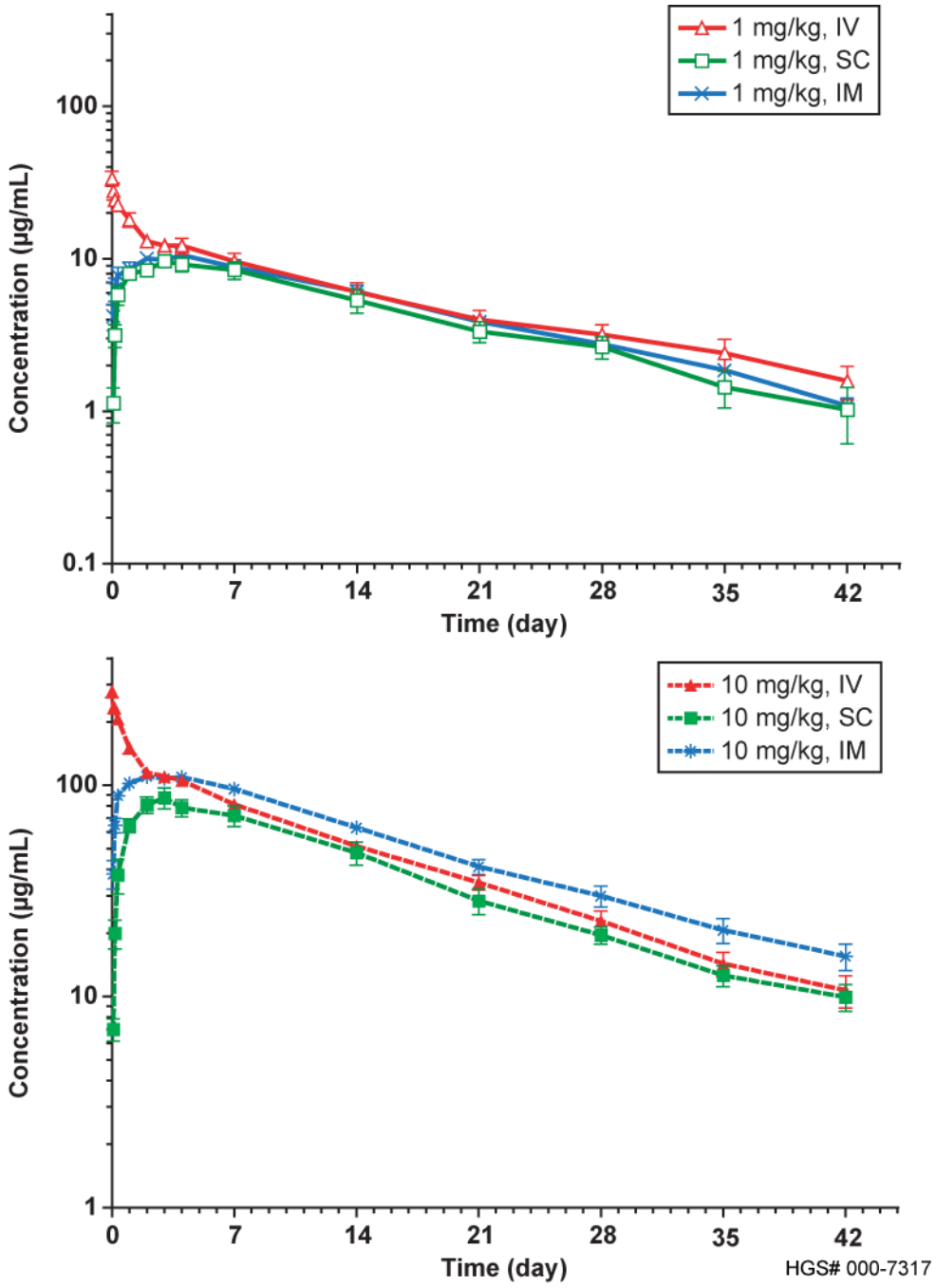

Figure 13-43 **Raxibacumab serum raxibacumab concentrations following a single IV, SC or IM injection of 1 mg/kg and 10 mg/kg raxibacumab in healthy cynomolgus monkeys (mean ± SD)**

13.15.1.3 Humans

Four clinical studies were conducted in healthy volunteers, which included evaluation of serum raxibacumab concentrations. Raxibacumab was 1st evaluated as a single dose administered IV or IM in a Phase 1 study, PAM-NH-01. Raxibacumab was subsequently evaluated as a single or repeat dose, 2 doses administered 14 days in HGS1021-C1063 or ≥ 4 months apart in HGS1021-C1069). Single raxibacumab doses were also evaluated alone or in combination with ciprofloxacin. Raxibacumab is intended for IV administration alone or in combination with antimicrobials.

13.15.1.3.1 Phase 1 Dose-Escalation Study (PAM-NH-01)

PAM-NH-01 was a Phase 1, single-blind, placebo-controlled, single-injection, dose-escalation study of raxibacumab in healthy subjects. The study was designed to evaluate the safety and PK of 3 single doses of IM administered raxibacumab (0.3, 1, and 3 mg/kg) and 5 single doses of IV administered raxibacumab (1, 3, 10, 20, and 40 mg/kg). Two injection sites were evaluated for the IM route of administration: gluteus maximus (0.3, 1, and 3 mg/kg) and vastus lateralis (1 and 3 mg/kg only). An IV infusion was administered over approximately 2 hours at a rate of 15 mL/hour for 0.33 hour, followed by 165 mL/hour for the remainder of the infusion. Raxibacumab Lots 03A21126, 03A21136, and 03A21142 were used in this study. Eligible subjects included males or females aged 18 to 65 years. Subjects using concomitant medications, with the exception of oral contraceptives, antihistamines, and nutritional supplements, were excluded.

In total, 105 subjects were randomized and treated (80 with raxibacumab and 25 with placebo) with 7-9 subjects in each raxibacumab treatment group and 2-3 subjects in each placebo group. The majority of the subjects in this study were male (66%) and Black/African American (69%), with an age range of 18 to 65 years, and body mass index (BMI) range of 19.0 to 54.3.

Blood samples for raxibacumab serum concentrations were collected at multiple times between Day 0 and 56 of the study. Serum raxibacumab concentrations were determined using a sandwich-type ELISA with a LLOQ of 200 ng/mL. Serum raxibacumab concentration-time data were analyzed using noncompartmental analysis. Descriptive statistics were used to calculate PK parameters and PK linearity was evaluated for each route and injection site using analysis of variance (ANOVA) or unpaired t-tests.

Serum raxibacumab concentrations following a single raxibacumab administration were detectable over the 56-day sampling period in most subjects. Mean PK parameters are presented in Table 13-36 for IV dosing and Table 13-37 for IM dosing. Mean raxibacumab serum concentrations for IV or IM administration are presented in and Figure 13-44, respectively. The mean terminal elimination half-life ($t_{1/2,z}$) ranged from 16.2 to 19.4 days after IV dosing and was similar for IM dosing (15.1 to 19.3 days). The t_{max} for an IM dose was approximately 6 days. CL was similar across doses within each route of administration. After IV infusion, the V_{ss} ranged from 58 to 73 mL/kg, indicating that the drug was not confined to the plasma volume (approximately 40 mL/kg) and was distributed to tissues. Raxibacumab PK is linear across the dose ranges evaluated by IV infusion or at either site of

IM injection. IM injection in the gluteus maximus was not equivalent to IM injection in the vastus lateralis as significantly lower dose-normalized AUC ($p = 0.0175$) and C_{max} ($p = 0.0159$) values were observed following injection in the gluteus maximus. Bioavailability was 71-85% following vastus lateralis injection and 50-54% following gluteus maximus injection.

Table 13-36 Raxibacumab PK parameters in healthy subjects following IV dosing with raxibacumab (PAM-NH-01)

Dose	1.0 mg/kg IV (N = 8)		3.0 mg/kg IV (N = 9)		10.0 mg/kg IV (N = 8[1])		20.0 mg/kg IV (N = 8)		40.0 mg/kg IV (N = 7)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
$t_{½}$ (h)	464.5	66.4	447.6	103.1	388.0	103.2	452.3	78.2	389.0	55.2
C_{max} (μg/mL)	25	5.356	72	17	226	25	481	92	1042	875
C_{max}/dose (kg/mL)	0.0255	0.0054	0.0241	0.0056	0.0226	0.0025	0.0241	0.0046	0.0261	0.0022
$AUC_{0-\infty}$ (μg/mL·h)	8817	1792	25772	5144819	71541	12271	173196	45541	373302	78564
AUC/dose (h/mL·kg)	8.82	1.79	8.59	1.71	7.15	1.23	8.66	2.28	9.33	1.96
CL (mL/kg/h)	0.12	0.020	0.12	0.02	0.14	0.02	0.12	0.03	0.11	0.02
V_z (mL/kg)	77.8	13.6	76.3	15.7	78.7	16.3	78.4	18.2	60.8	5.5
V_{ss} (mL/kg)	69.5	10.6	70.8	14.1	72.5	14.5	71.5	16.1	57.6	5.2
MRT_{IV} (h)	600.1	77.9	597.0	122.3	517.1	142.9	596.8	115.4	532.8	87.6

[1] C_{max} was determined for all subjects. Terminal phase parameters could not be calculated for 1 subject (US001-000053) in the 10 mg/kg dose group.

Table 13-37 PK parameters in healthy subjects following IM dosing with raxibacumab (PAM-NH-01)

Dose (site[1])	0.3 mg/kg (G) (N = 8[2])		1.0 mg/kg (G) (N = 8[2])		3.0 mg/kg (G) (N = 8)		1.0 mg/kg (V) (N = 8[2])		3.0 mg/kg (V) (N = 8)	
$t_{½}$ (h)	463.3	78.9	376.6	100.1	362.8	56.5	427.9	250.3	432.8	127.7
t_{lag} (h)[3]	4	0-58	0	0-3	0	0-2	0	0-2	0	0-2
t_{max} (h)[3]	168	72-316	144	12-342	96	72-173	144	48-173	120	72-336
C_{max} (μg/mL)	2.0	1.3	8.0	3.0	22.5	7.1	9.8	1.6	28.5	6.6
C_{max}/dose (kg/mL)	0.0066	0.0043	0.0080	0.0030	0.0075	0.0023	0.0098	0.0016	0.0095	0.0022
$AUC_{0-\infty}$ (μg/mL·h)	1811	766	4731	1589	12810	4322	6267	3064	21891	7216
AUC/dose (h/mL·kg)	6.04	2.55	4.73	1.59	4.27	1.44	6.27	3.06	7.30	2.41
V_z/F (mL/kg)	128	53	140	111	135	49	97	18	87	14
CL/F (mL/kg/h)	0.21	0.12	0.25	0.14	0.26	0.10	0.21	0.13	0.15	0.05
MRT_{IM} (h)	704.6	101.3	568.8	150.9	569.1	99	646.7	362.6	678.7	186.6

1 IM injection site: G (gluteus maximus), V (vastus lateralis).

2 C_{max} and t_{max} were determined for all subjects. Terminal phase parameters could not be calculated for 1 subject in the 1 mg/kg (G) and 2 subjects in each of the 0.3 mg/kg (G) and 1 mg/kg (V) groups.

3 t_{lag} and t_{max} are summarized as median and range.

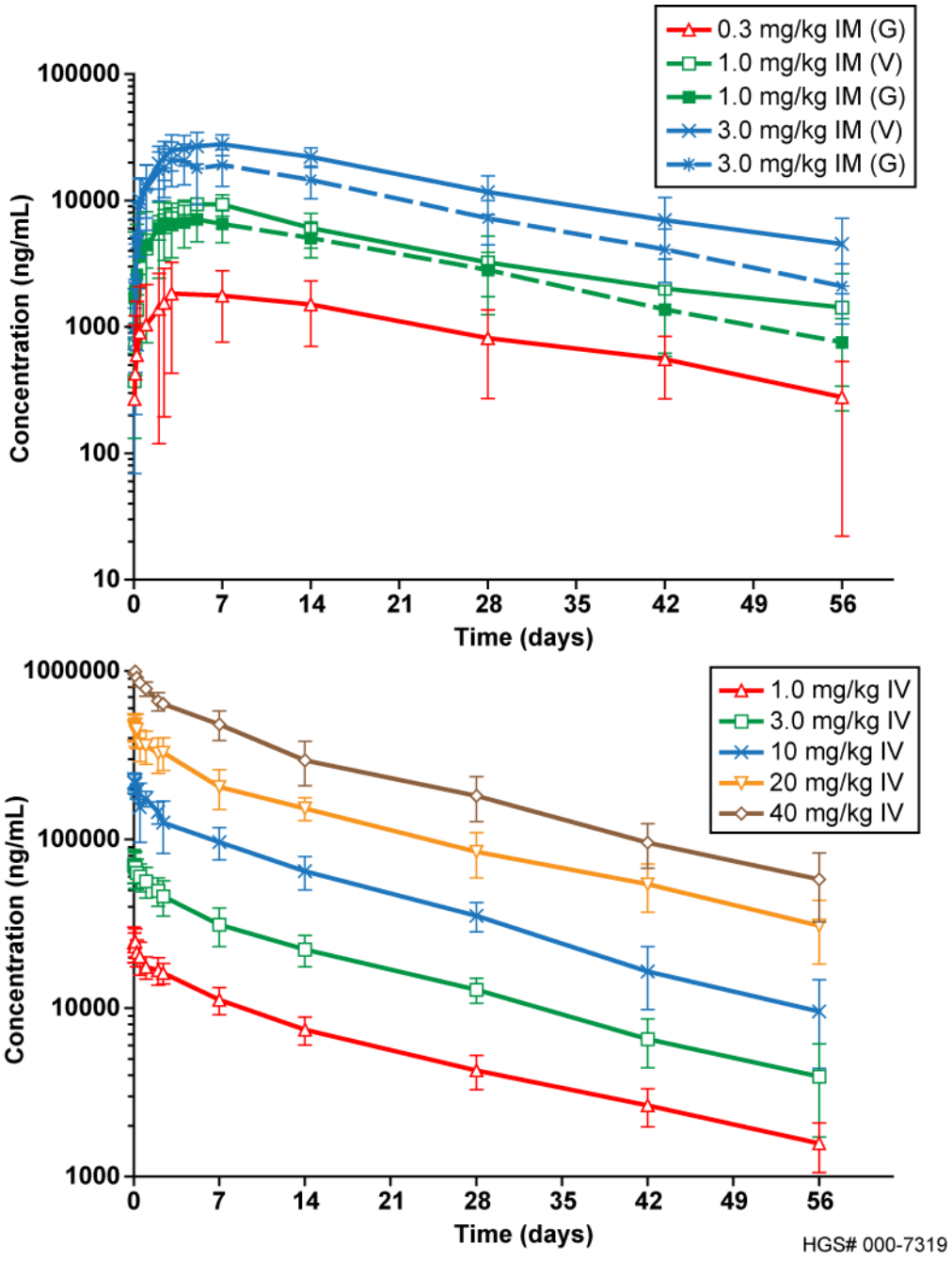

Figure 13-44 Raxibacumab serum concentrations in healthy subjects administered 0.3 to 3 mg/kg raxibacumab by IM (mean ± SD) (top graph) and administered 1 to 40 mg/kg raxibacumab (mean ± SD) (bottom graph)

Raxibacumab was administered IM in either the gluteus maximus (G) or vastus lateralis (V).

13.15.1.3.2 Phase 3 Safety Study (HGS1021-C1063)

This was a randomized, single-blind, placebo-controlled study of raxibacumab to evaluate the safety and tolerability of IV administered raxibacumab in healthy subjects and to determine serum raxibacumab concentrations for use in a population PK analysis. Subjects were to be randomized to 1 of 2 raxibacumab groups (40 mg/kg double dose or 40 mg/kg single dose) or to 1 of 2 matching placebo groups at a ratio of 3:1 (raxibacumab:placebo). Subjects in the double-dose cohorts received doses of raxibacumab or placebo on Days 0 and 14, while subjects in the single dose cohorts were administered treatment on Day 0. All subjects were to be administered PO 50 mg diphenhydramine within 1 hour prior to treatment. Subjects were stratified at randomization by age (< age 65 or ≥ age 65). Raxibacumab Lots 71044 and 71051 were used for this study.

In total, 320 subjects were treated (74 in the placebo single-dose group, 6 in the placebo double-dose group, 217 in the raxibacumab single-dose group, and 23 in the raxibacumab double-dose group). The raxibacumab-treated study population included approximately 9% of subjects ≥ 65 years of age (range: 18 to 88 years) and 2.5% ≥ 75 years of age, 54% females, 12% Hispanic, and 21% non-white with a weight range of 45 to 156 kg. Of these, 318 subjects were considered evaluable for raxibacumab PK.

Blood samples for serum raxibacumab concentration measurement were collected from all subjects in the single dose cohorts prior to treatment on Day 0, at 30 minutes and 2 to 6 hours after completion of the raxibacumab infusion, and at 14, 28, and 56 days after the raxibacumab dose. For the double-dose cohorts, blood samples were collected prior to treatment on Days 0 and 14, at 30 minutes and 2 to 6 hours after completion of each raxibacumab infusion, and at 28, 42, 56, and 70 days after the 1st raxibacumab dose. Serum samples were analyzed for raxibacumab using an ECL-based assay. The LLOQ is 800 ng/mL of raxibacumab in 100% serum.

Serum raxibacumab concentration-time results for the raxibacumab single dose and double-dose groups are illustrated in Figure 13-45. For comparison, the figure also shows the serum raxibacumab concentration-time profile following a single 40 mg/kg IV infusion of raxibacumab in Protocol HGS1021-C1064. The serum raxibacumab concentrations observed in this study are consistent with raxibacumab PK observed in the prior study. Most raxibacumab-treated subjects in this study had measurable raxibacumab concentrations for up to 56 days post-treatment. Following the 2nd raxibacumab dose, accumulation was observed as could be expected given serum raxibacumab concentrations were still measurable at Day 14.

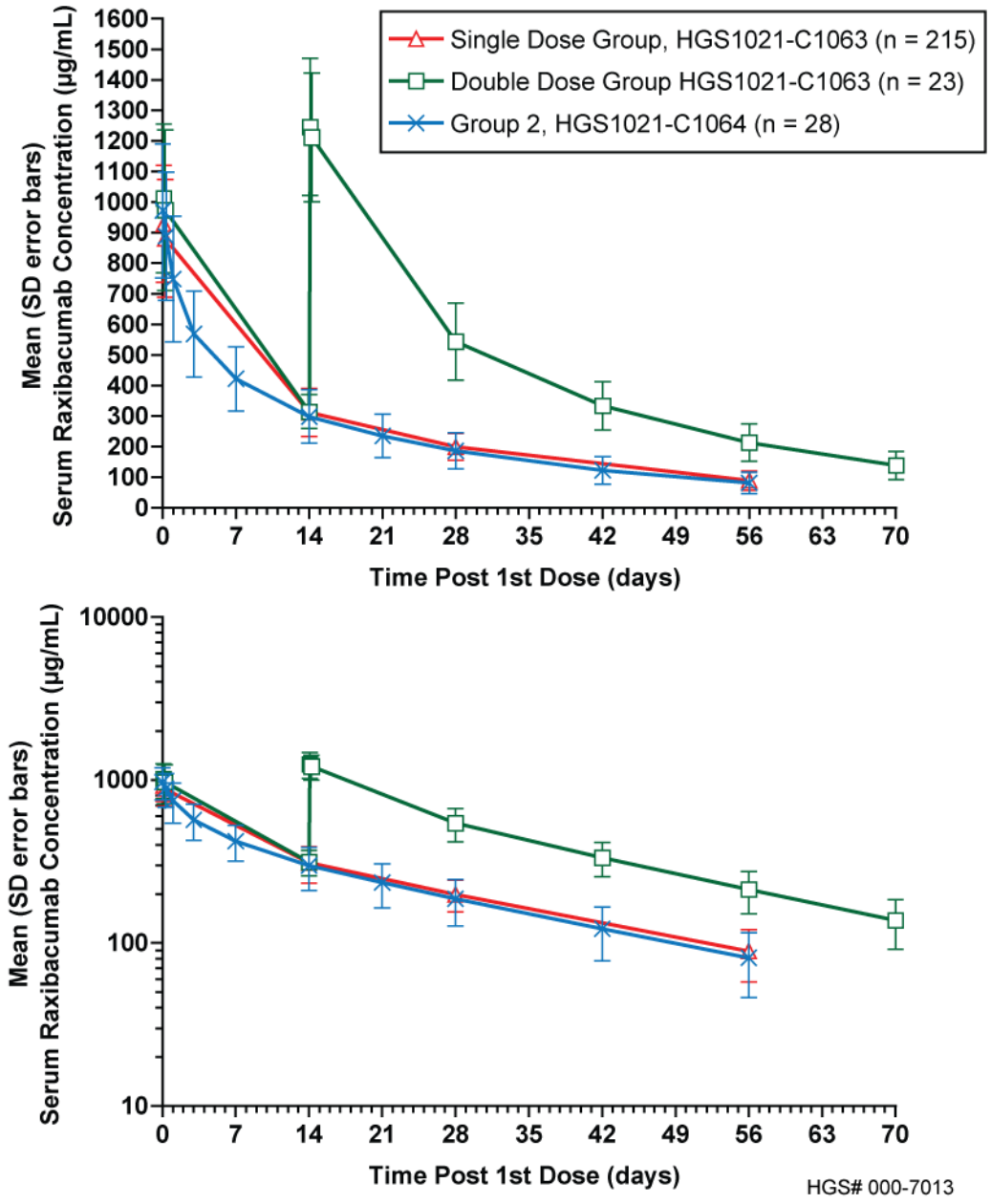

Figure 13-45 Raxibacumab serum concentration-time profiles in healthy subjects (mean ± SD) (HGS1021-C1063)

Top panel – linear plot; bottom panel – semi log plot. Subjects in Study HGS1021-C1063 received 40 mg/kg raxibacumab IV on Day 0 (single dose) or on Days 1 and 14 (double dose). Subjects receiving a single dose of 40 mg/kg raxibacumab IV from a prior study (HGS1021-C1064) are shown for comparison.

13.15.1.3.3 Phase 2/3 Repeat-Dose Immunogenicity Study (HGS1021-C1069)

This was an open-label study to evaluate the immunogenicity, safety, and PK of a 2nd dose of raxibacumab administered after a wash-out period in healthy adult male and female subjects. A single 40 mg/kg IV raxibacumab dose (Lot 71044) was administered on Day 0 to all subjects in this study. All subjects had received a single 40 mg/kg IV dose of raxibacumab in a previous study at least 4 months prior (mean 7.6 ± 0.7 months prior) to dosing in this study.

Twenty subjects were treated with 40 mg/kg IV raxibacumab and were all considered evaluable for PK. There were a similar number of male and female subjects in this study (12 males and 8 females). The majority of subjects in this study were White (13/20, 65%) with representation of African Americans 7/20 (35%). The age range was 23 to 61 years and weight ranged from 56 to 101 kg.

Blood samples for serum raxibacumab concentration measurement were collected from all subjects just prior to treatment, at 5 minutes and 8 hours after raxibacumab infusion, and at 1, 3, 7, 14, 21, 28, 42, and 56 days after dosing. Serum samples were analyzed for raxibacumab using the same ECL-based assay. Serum raxibacumab concentration-time data were analyzed using noncompartmental techniques to determine PK parameters. Raxibacumab PK parameters for the 2nd dose (administered in the current study) were summarized with descriptive statistics. To evaluate potential differences, the raxibacumab PK results for the 1st dose (administered ≥ 4 months prior) were compared with the 2nd dose using paired t-tests with the exception of t_{max} for which the Wilcoxon matched pairs test was used (Table 13-38).

Serum raxibacumab concentration-time profiles were very similar for 2 doses of raxibacumab administered at least 4 months apart (Figure 13-46).

Table 13-38 Raxibacumab PK parameters after 2nd dose after ≥ 4 months (HGS1021-C1069)

Parameter	Current Study (HGS1021-C1069) (2nd Dose) (n = 20)	Prior Study (HGS1021-C1064) (1st Dose) (n = 20)
C_{max} (µg/mL)	979 ± 148 (p = 0.0008[1])	1152 ± 176
C_{max}/Dose (kg/mL)	0.0245 ± 0.0037 (p = 0.0011[1])	0.0290 ± 0.0047
t_{max} (day)[2]	0.099 (0.097 to 0.104) (p = 0.0019[1])	0.102 (0.097 to 0.431)
$AUC_{0-\infty}$ (µg·day/mL)	18239 ± 6179 (p = 0.1798[1])	16440 ± 4140
$AUC_{0-\infty}$/Dose (kg·day/mL)	0.4566 ± 0.1538 (p = 0.1853[1])	0.4122 ± 0.1017
$t_{1/2,z}$ (day)	25.68 ± 11.19 (p = 0.1535[1])	21.20 ± 8.62

Table 13-38 Raxibacumab PK parameters after 2nd dose after ≥ 4 months (HGS1021-C1069)

Parameter	Current Study (HGS1021-C1069) (2nd Dose) (n = 20)	Prior Study (HGS1021-C1064) (1st Dose) (n = 20)
MRT (day)	35.09 ± 15.58	27.21 ± 8.62
	(p = 0.0594[1])	
CL (mL/day/kg)	2.37 ± 0.63	2.59 ± 0.77
	(p = 0.3017[1])	
V_{ss} (mL/kg)	75.72 ± 11.42	64.73 ± 14.02
	(p = 0.0122[1])	
V_z (mL/kg)	80.07 ± 12.33	72.60 ± 18.96
	(p = 0.1425[1])	

Abbreviations: C_{max}, maximum serum raxibacumab concentration for a single dose; t_{max}, time of occurrence for C_{max}; $AUC_{0-\infty}$, area under the serum raxibacumab concentration-time curve from time 0 to infinite time for a single dose; $t_{1/2,z}$, elimination half-life for the terminal phase; MRT, mean residence time; CL, clearance; V_{ss}, volume of distribution at steady-state; V_z, volume of distribution in the terminal phase.

1 P-value from a paired t-test; the exception is t_{max}, for which the p-value is from a Wilcoxon matched pairs test.

2 Median and range are presented.

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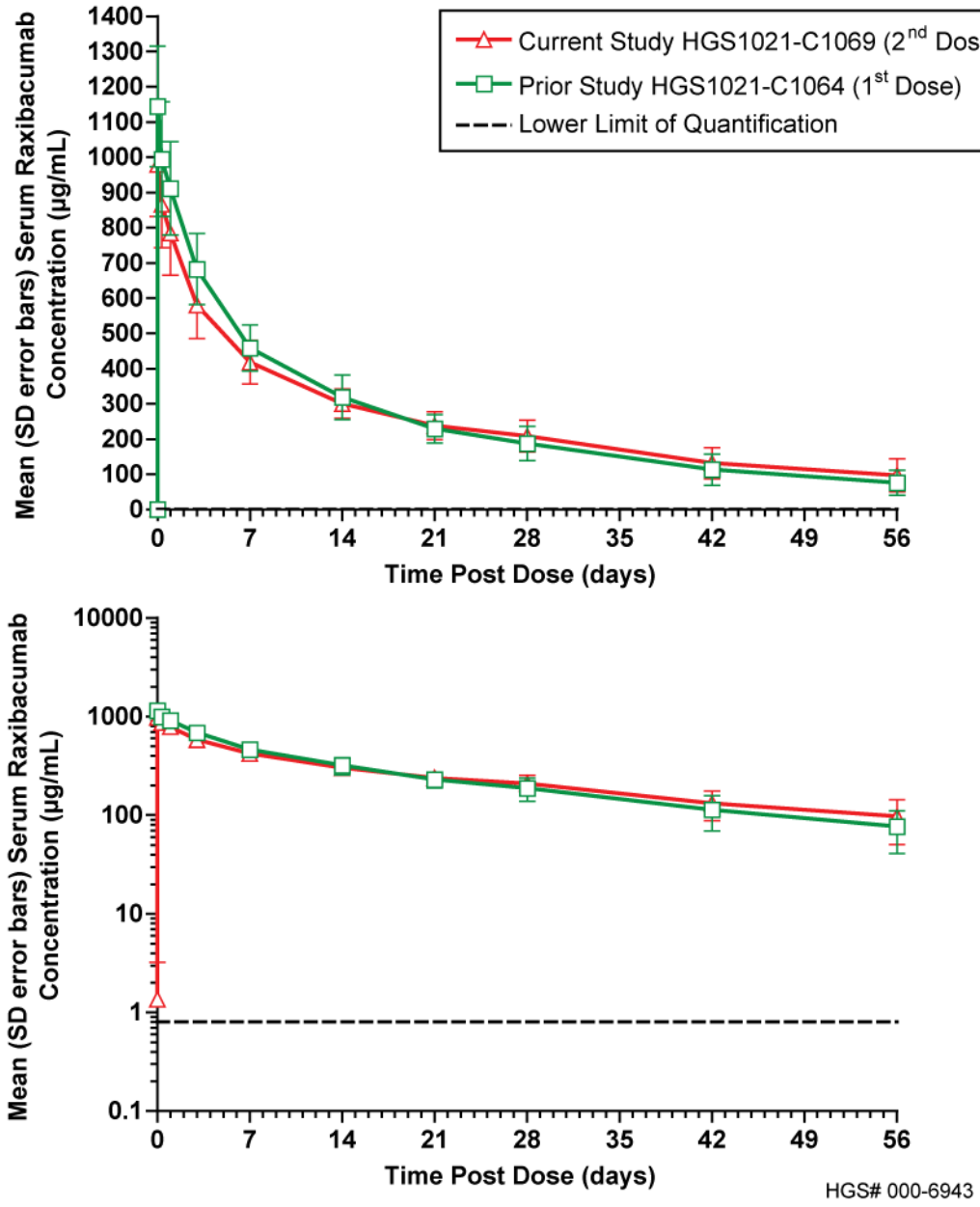

Figure 13-46 Raxibacumab serum concentrations following 40 mg/kg raxibacumab IV infusion doses, administered at least 4 months apart (mean ± SD) (HGS1021-C1069)

Top panel – linear plot; bottom panel – semi log plot. Subjects in Study HGS1021-C1069 received a single dose of 40 mg/kg raxibacumab IV at least 4 months following treatment with a single dose of 40 mg/kg raxibacumab IV in Study HGS1021-C1064. Serum concentrations from both doses are shown for comparison.

13.15.2 Raxibacumab Pharmacokinetics in Inhalation Anthrax-Spore Challenged Rabbits and Monkeys

In addition to PK evaluation in healthy humans and animals, raxibacumab PK were evaluated in anthrax spore-challenged rabbits and monkeys as part of 2 prophylactic efficacy studies as well as in the therapeutic efficacy studies.

13.15.2.1 Rabbit Pre-exposure Prophylactic Study (Study 288-HGSIRAB) (AB50409.INF.0.027)

The PK of raxibacumab were evaluated in anthrax spore-challenged rabbits (Report AB 50409.INF.0.027) when raxibacumab was administered as a prophylactic or immediate PEP therapeutic agent as part of Study 288-HGSIRAB.

In total, 72 New Zealand white (NZW) rabbits weighing 2.5 to 3.2 kg were randomly assigned to 1 of 6 groups with 12 animals per group (6 male, 6 female/group). Animals received a single SC dose of raxibacumab (Lot 02A21109) administered in the mid-scapular region at 0 (vehicle) 1, 5, 10, or 20 mg/kg 2 days prior to anthrax spore challenge. In addition, 1 group received a single 40 mg/kg IV raxibacumab dose administered within 1 hour following spore challenge. Rabbits were aerosol challenged with a targeted dose of 100 x LD_{50} *B. anthracis* spores (Ames strain).

Blood samples for raxibacumab serum analysis were collected from all rabbits predose (1 week prior to spore challenge) and at 1, 2, 4, and 14 days following anthrax exposure. If possible, a blood sample was obtained from any animal found moribund, regardless of scheduled time for sample collection. Serum samples were analyzed for raxibacumab using the same ELISA format as described above for Study AB50409.INF.0.016.

PK analyses were conducted by noncompartmental methods (Table 13-39). Serum raxibacumab concentration-time data and PK parameters were summarized using the descriptive statistics. An unpaired t-test was used to compare raxibacumab serum concentrations at corresponding times and raxibacumab PK between the spore-challenged animals in the current study with healthy rabbits in a prior study.

In the SC dose groups, serum raxibacumab concentrations were highest on the 3rd day following SC administration and showed a gradual decline up to 16 days post raxibacumab administration (Figure 13-47). These observations are consistent with the previous study in healthy rabbits showing that t_{max} occurs 2-3 days following SC dosing (Report AB50409.INF.0.016).

Table 13-39 Raxibacumab PK in rabbits (PEP) (288-HGSIRAB)

Parameter	1 mg/kg SC (n = 11)	5 mg/kg SC (n = 12)	10 mg/kg SC (n = 12)	20 mg/kg SC (n = 12)	40 mg/kg IV (n = 12)
C_{max} (µg/mL)	10.66	52.38	102.03	191.66	787.24
95% CI	9.71, 11.61	47.76, 57.00	89.95, 114.10	175.11, 208.21	528.54, 731.41
C_{max}/Dose (kg/mL)	0.0107	0.0105	0.0102	0.0096	0.0197
95% CI	0.0078, 0.0147	0.0092, 0.0114	0.0090, 0.0114	0.0088, 0.0104	0.0164, 0.0230
AUC_{0-4d} (µg·day/mL)	24.90	126.75	245.09	460.00	2076.00
95% CI	0.0078, 0.0147	115.44, 138.05	216.00, 274.18	420.25, 499.75	1765.25, 2386.76
AUC_{0-4d}/Dose (kg·day/mL)	0.0249	0.0253	0.0245	0.0230	0.0519
95% CI	0.0221, 0.0277	0.0231, 0.0276	0.0216, 0.0274	0.0210, 0.0250	0.0441, 0.0597

Abbreviations: C_{max}, maximum serum raxibacumab concentration; AUC_{0-4d}, area under the serum concentration time curve for the 4 days following dose administration.

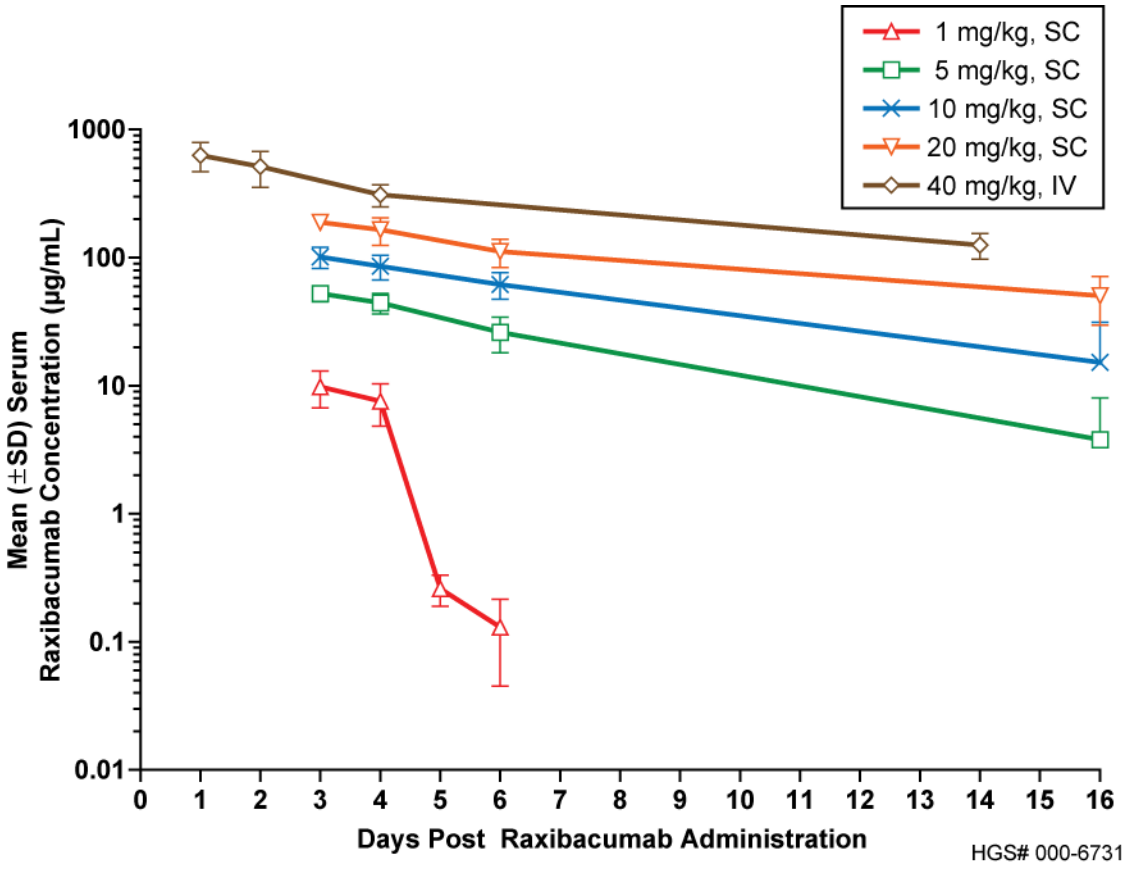

Figure 13-47 Raxibacumab serum concentration-time profiles in rabbits (mean ± SD) (PEP) (288-HGSIRAB)

13.15.2.2 Rabbit Therapeutic Treatment Study (Study 682-G005758) (AB50409.INF.0.036)

The PK of raxibacumab (Report AB50409.INF.0.036) was evaluated in rabbits symptomatic for inhalation anthrax as part of the Good Laboratory Practices (GLP) pivotal efficacy study for the therapeutic treatment indication (Study 682-G005758.

In total, 54 NZW rabbits (29 males and 25 females) weighing 2.90 to 3.97 kg were randomized by gender and body weight into 3 treatment groups. All rabbits were aerosol challenged with a target of 200 x LD_{50} *B. anthracis* spores on Day 0. Following detection of serum PA or body temperature increase, rabbits were treated with a single dose of 20 or 40 mg/kg raxibacumab or placebo. One rabbit died prior to study initiation, and thus a total of 53 rabbits were placed on study. In addition, 1 rabbit randomized to the control group was treated with 40 mg/kg raxibacumab.

Blood samples were collected 3 days prior to spore challenge; just prior to dosing; and at 5 minutes as well as at 4, 10, 24, 36, 48, 72, 144, and 216 hours after dosing. Serum samples were analyzed for raxibacumab using an ECL-based assay. The LLOQ is 750 ng/mL of raxibacumab in 100% rabbit serum. Immunogencity was not measured in this study because of the 14-day duration of the index study period, which was thought too short to allow development of an immune response.

PK analyses of raxibacumab concentration-time profiles for all raxibacumab-dosed rabbits were conducted using population analysis techniques (mixed effect modeling). Both 2- and 3-compartment models were assessed, and the serum raxibacumab concentrations best fit a 2-compartment open model with 1st-order elimination from the central compartment. Inter-individual variability for CL, V_1, and volume of distribution for the peripheral (2nd) compartment (V_2) were modeled using exponential terms. Inter-individual variability for intercompartmental clearance (CLD_2) was not modeled. Body weight was found to be a significant covariate for V_2. The factors assessed (body weight, sex, treatment group, size of spore challenge, and survival status) were not significant covariates accounting for inter-individual differences in PK, other than as mentioned previously for weight and V_2. The lack of difference in PK between treatment groups is consistent with linear PK over the dose range studied.

The individual observed serum raxibacumab concentrations versus the predicted population average serum raxibacumab concentration-time profiles for this study are presented in Figure 13-48 and the parameter estimates for the PK model are summarized in Table 13-40. Overall, these results indicate that there is minimal variability in raxibacumab disposition, even in rabbits exhibiting symptoms of inhalation anthrax.

Table 13-40 Raxibacumab PK parameters in rabbits (682-G005758)

Primary Parameters	Mean	CV%
V_1 (mL)	132	15.1
CL (mL/day)	35	18.5
V_2 (mL)	57[1]	27.6
Effect of weight on V_2	$V_2 = 56.922 + (63.477 \times [\text{weight} - 3])$	
At 2.5 kg	25	
At 2.75 kg	41	
At 3.25 kg	73	
At 3.5 kg	89	
CLD_2 (mL/day)	112	NA
Secondary Parameters	**Mean for 20 mg/kg**	**Mean for 40 mg/kg**
C_{max} (µg/mL)[1]	455	909
$AUC_{0-\infty}$ (µg·day/mL)[1]	1706	3412
$t_{1/2,\alpha}$ (days)	0.24	0.24
$t_{1/2,\beta}$ (days)	3.84	3.84
MRT (days)	5.37	5.37
V_{ss} (mL)[1]	189	189

Abbreviations: CV%, coefficient of variation; V_1, volume of distribution for the central compartment; CL, clearance; V_2, volume of distribution for the peripheral compartment; CLD_2, intercompartmental clearance; NA, not applicable; C_{max}, maximum serum drug concentration; $AUC_{0-\infty}$, area under the serum drug concentration-time curve from time 0 to infinite time; $t_{1/2,\alpha}$, elimination half-life for the 1st phase; $t_{1/2,\beta}$, elimination half-life for the 2nd (terminal) phase; MRT, mean residence time; V_{ss}, volume of distribution at steady-state.

[1] Assuming a typical rabbit weighing 3 kg.

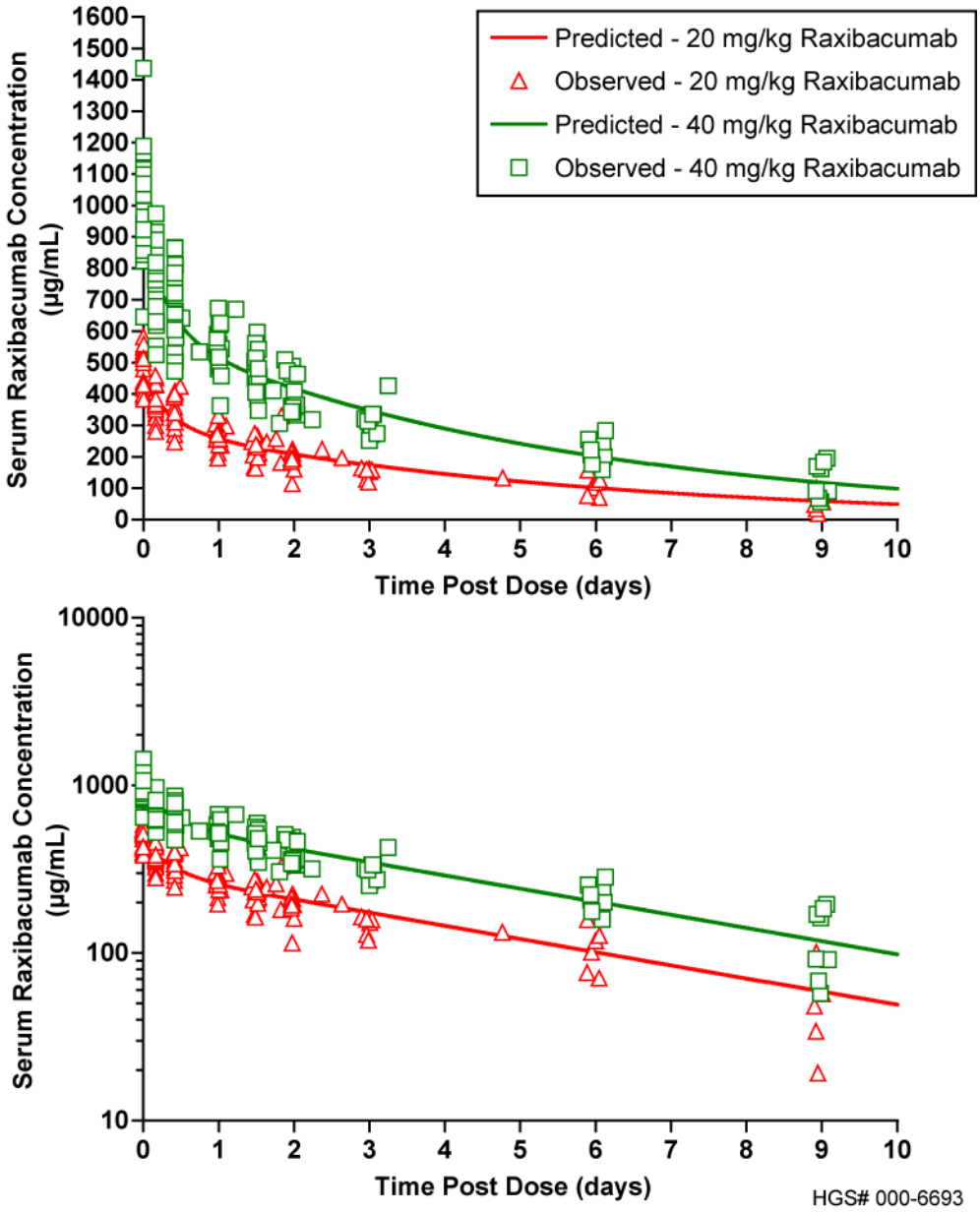

Figure 13-48 Predicted population average serum raxibacumab concentration-time profiles and individual observed serum raxibacumab concentrations in rabbits (682-G005758)

Upper panel, linear scale; lower panel, semilog scale.

13.15.2.3 Monkey Pre-exposure Prophylactic Study (Study 290-N005433) (AB50409.INF.0.028)

The PK of raxibacumab (Report AB50409.INF.0.028) were assessed as part of Study 290-N005433) in cynomolgus monkeys administered raxibacumab as a pre-exposure prophylactic treatment.

In total, 40 cynomolgus monkeys weighing 1.9 to 2.8 kg were randomized into 4 groups of 10 animals each (5 male, 5 female/group). Monkeys were treated with a single SC dose of 0 (vehicle), 10, 20, or 40 mg/kg raxibacumab administered in the mid-scapular region. All monkeys were aerosol challenged with a targeted dose of 100 x LD_{50} *B. anthracis* (Ames strain) spores 48 hours after treatment administration.

Blood samples were collected for serum raxibacumab concentrations from all monkeys predose, 2 days post dose (immediately prior to spore challenge), 9, 16, and 30 days post treatment. Serum samples were analyzed for raxibacumab using the same ELISA format as described above for Study AB50409.INF.0.017 PK analyses were conducted by noncompartmental methods and raxibacumab concentration-time data and PK parameters were summarized using descriptive statistics.

The peak raxibacumab serum concentrations of raxibacumab were observed 2 days following raxibacumab administration, with a gradual decline in serum concentration to 30 days post dose (Figure 13-49). These observations are consistent with a previous study in normal monkeys showing that t_{max} occurs between 2-3 days following a single SC dose of raxibacumab (Report AB50409.INF.0.017). A summary of the PK parameters is shown in Table 13-41. There are no significant differences in PK parameters between the 3 treatment groups and dose proportional increases in C_{max} and $AUC_{0-\infty}$ are observed across the dose range used in this study.

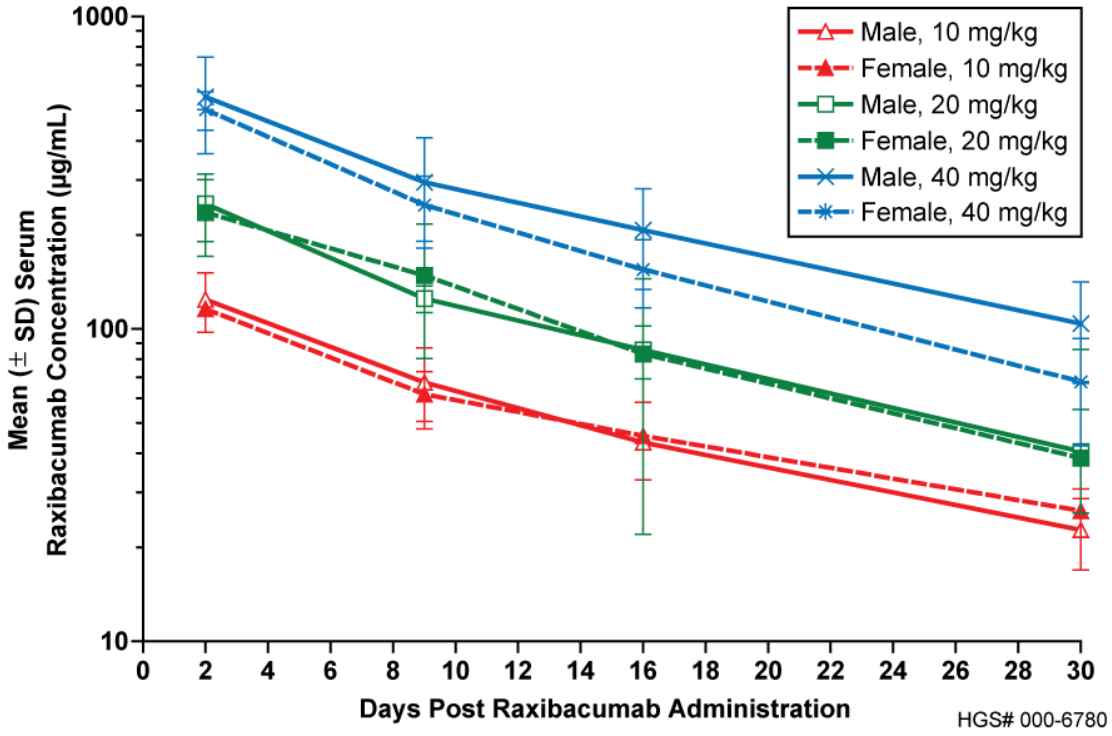

Figure 13-49 **Raxibacumab serum concentration-time profiles in male and female monkeys (mean ± SD)**

Table 13-41 **Raxibacumab PK parameters in monkeys (Study 290-N005433)**

Parameter	Raxibacumab 10 mg/kg (n = 6)	Raxibacumab 20 mg/kg (n = 6)	Raxibacumab 40 mg/kg (n = 6)
C_{max} (µg/mL)	120.2	243.8	528.4
95% CI	(106.7, 133.7)	(200.9, 286.7)	(430.4, 626.4)
C_{max}/Dose (kg/mL)	0.0120	0.0122	0.0132
95% CI	(0.0107, 0.0134)	(0.0100, 0.0143)	(0.0108, 0.0157)
$AUC_{0-\infty}$ (µg·day/mL)	2217	3953	8045
95% CI	(1870, 2564)	(2669, 5237)	(6089, 10001)
$AUC_{0-\infty}$/Dose (kg·day/mL)	0.2217	0.1976	0.2011
95% CI	(0.1870, 0.2564)	(0.1334, 0.2618)	(0.1522, 0.2500)
t_{max} (day)[1]	2.0[2]	2.0[2]	2.0[2]
Min. to max.	(All values = 2.0)	(All values = 2.0)	(All values = 2.0)
$t_{1/2\ term}$ (day)	17.0	12.1	12.7

Table 13-41 Raxibacumab PK parameters in monkeys (Study 290-N005433)

Parameter	Raxibacumab 10 mg/kg (n = 6)	Raxibacumab 20 mg/kg (n = 6)	Raxibacumab 40 mg/kg (n = 6)
95% CI	(9.5, 24.4)	(8.1, 16.1)	(10.4, 15.1)
CL/F (mL/day/kg)	4.6	5.7	5.4
95% CI	(3.9, 5.3)	(3.6, 7.8)	(4.2, 6.5)
V_z/F (mL/kg)	109.0	88.7	95.5
95% CI	(72.2, 145.8)	(74.8, 102.6)	(75.3, 115.7)

Abbreviations: C_{max}, maximum serum raxibacumab concentration; CI, confidence interval; $AUC_{0-\infty}$, area under the serum concentration time curve from time 0 to infinite time; t_{max}, time of maximum serum raxibacumab concentration; min., minimum value; max., maximum value; $t_{1/2,term}$, terminal half-life; CL/F, apparent clearance after SC dosing; V_z/F, apparent volume of distribution in the terminal phase after SC dosing.

[1] Median and minimum to maximum value reported for t_{max}.

[2] For this PK parameter, n = 10.

(concluded)

13.15.2.4 Monkey Therapeutic Treatment Study (724-G005829) (AB50409.INF.0.040)

The PK of raxibacumab (Report AB50409.INF.0.040) was evaluated in monkeys symptomatic for inhalation anthrax as part of the GLP confirmatory efficacy study for the therapeutic treatment indication (Study 724-G005829).

In total, 40 cynomolgus monkeys (20 males and 20 females) weighing 2.3 to 5.1 kilograms were randomized by gender into 3 treatment groups. All monkeys were aerosol challenged with a targeted 200 x LD_{50} inhaled dose of *B. anthracis* (Ames strain) spores on Day 0. Following detection of measurable serum PA, monkeys were treated with a single dose of 20 or 40 mg/kg raxibacumab or placebo administered IV. Blood samples were to be collected from all monkeys at 3 days prior to spore challenge; just prior to dosing; at 5 minutes after dosing; at 12 and 24 hours after dosing; and at 3, 5, 8, 14, and 28 days after dosing. Serum samples were analyzed for raxibacumab using an ECL-based assay. The LLOQ is 940 ng/mL of raxibacumab in 100% monkey serum.

PK analyses of raxibacumab concentration-time profiles for all raxibacumab-dosed monkeys were conducted using population analysis techniques (mixed effect modeling). Both 2- and 3-compartment models were assessed, and the serum raxibacumab concentrations best fit a 2-compartment open model with 1st-order elimination from the central compartment. CL, V_1, and V_2 were modeled using exponential terms. CLD_2 was not modeled. Body weight was found to be a significant covariate for V_2, while sex was a significant covariate for V_1 and CLD_2. Other factors assessed (age, treatment group, size of spore challenge, duration of spore challenge, survival time, survival status, time to 1st bacteremia by culture, and bacteremia outcome at each collection time) were not significant covariates accounting for inter-individual differences in PK. The lack of difference in PK between treatment groups is consistent with linear PK over the dose range studied. The parameter estimates for the PK

model are summarized in Table 13-42. Overall, these results indicate that there is minimal variability in raxibacumab disposition, even in monkeys exhibiting symptoms of inhalation anthrax.

Table 13-42 Raxibacumab PK parameters in monkeys (724-G005829)

Primary Parameters	Mean	CV%
V_1 (mL)	160	15.8
Effect of sex on V_1 (mL)[1]	V_1 = 159.8 x (1 + (-0.25202 x sex))	
Males	160	
Females	120	
CL (mL/day)	20	25.9
V_2 (mL)	125[1]	16.0
Effect of weight on V_2 (mL)	V_2 = 125.02 + (43.545 x (weight –3))	
At 2.5 kg	103	
At 2.75 kg	114	
At 3.25 kg	136	
At 3.5 kg	147	
CLD_2 (mL/day)	87	NA
Effect of sex on CLD_2 (mL/day)[1]	CLD_2 = 86.789 x (1 + (-0.44941 x sex))	
Males	87	
Females	48	

	Mean for 20 mg/kg		Mean for 40 mg/kg	
Secondary Parameters	**Males**	**Females**	**Males**	**Females**
C_{max} (µg/mL)[2]	375	751	502	1004
$AUC_{0-\infty}$ (µg·day/mL)[2]	2966	5931	2966	5931
$t_{1/2,\alpha}$ (days)	0.53	0.79	0.53	0.79
$t_{1/2,\beta}$ (days)	10.22	9.40	10.22	9.40
MRT (days)	14.08	12.09	14.08	12.09
V_{ss} (mL)	285	245	285	245

Abbreviations: CV%, coefficient of variation; V_1, volume of distribution for the central compartment; CL, clearance; V_2, volume of distribution for the peripheral compartment; CLD_2, intercompartmental clearance; NA, not applicable; C_{max}, maximum serum drug concentration; $AUC_{0-\infty}$, area under the serum drug concentration-time curve from time 0 to infinite time; $t_{1/2,\alpha}$, elimination half-life for the 1st phase; $t_{1/2,\beta}$, elimination half-life for the 2nd (terminal) phase; MRT, mean residence time; V_{ss}, volume of distribution at steady-state.

1 For sex coded as 0 = male and 1 = female.

2 Assuming a typical monkey weighing 3 kg.

13.15.3 Pharmacokinetics (Drug-Drug Interaction Studies)

The PK of raxibacumab was evaluated in combination with antimicrobials in rabbits, monkeys and humans to determine whether raxibacumab or antimicrobial PK was affected when used concomitantly.

13.15.3.1 Pilot Study of Levofloxacin Pharmacokinetics (Study 723-G005835) (AB50409.INF.0.039.2)

A pilot study was undertaken to evaluate the PK of levofloxacin (Report AB50409.INF.0.039.2) in rabbits with inhalation anthrax in the therapeutic treatment setting to determine the appropriate levofloxacin dose for use in the raxibacumab and levofloxacin combination study (Study 723-G005835).

In total, 27 NZW rabbits (15 male, 12 female) weighing 3.2 to 4.25 kg were randomized by gender and weight into 3 levofloxacin treatment groups of 8 animals each (4 male, 4 female). The 3 remaining rabbits served as a control group. On Day 0, all rabbits were aerosol challenged with a targeted 200 x LD_{50} inhaled dose of *B. anthracis* (Ames strain) spores. Following detection of temperature increase, rabbits were treated with 10, 25, or 50 mg/kg levofloxacin administered orally by gastric intubation (GI) once daily (qd) for 3 days.

Blood specimens for plasma levofloxacin PK were collected just prior to administration of the 1st levofloxacin dose, and at the following times after the 1st levofloxacin dose: 0.25, 0.5. 1, 1.5, 2, 2.5, 3, 5, 9, 12, 23.75 (prior to the 2nd levofloxacin dose), 26.5, 47.75 (prior to the 3rd levofloxacin dose), 50.5, 71.75, and 96 hours. Plasma samples were analyzed for levofloxacin using a high performance liquid chromatography (HPLC)/fluorescence detection assay. The calibration range for the assay was from 40.53 to 15199.00 ng/mL. Plasma levofloxacin concentration-time results were analyzed by noncompartmental methods and summarized using descriptive statistics.

Plasma levofloxacin concentration-time profiles are illustrated in Figure 13-50 and levofloxacin PK parameters are summarized in Table 13-43.

A goal of obtaining levofloxacin PK results in this study was to allow identification of a levofloxacin dose that would result in exposure (C_{max} and AUC) similar to that for humans administered the recommended levofloxacin doses, 500 or 750 mg. In this study, the 50 mg/kg levofloxacin dose provided exposures intermediate to the human exposures at 500 and 750 mg. Therefore, the 50 mg/kg levofloxacin dose in rabbits was selected to target similar exposure to that for humans administered 500 or 750 mg of levofloxacin in the raxibacumab/levofloxacin interaction study in rabbits.

Table 13-43 Levofloxacin PK parameters (mean ± SD) for rabbits administered PO levofloxacin (723-G005835)

	Levofloxacin		
Parameter	**10 mg/kg (n = 8)**	**25 mg/kg (n = 8)**	**50 mg/kg (n = 8)**
t_{lag} (h) [1]	0.00 (0.00 to 0.55)	0.00 (0.00 to 0.42)	0.00 (0.00 to 0.35)
C_{max} (ng/mL)	1408 ± 341	3466 ± 894	8737 ± 4595
C_{max}/Dose (kg/L)	0.1408 ± 0.0341	0.1386 ± 0.0358	0.1747 ± 0.0919
t_{max} (h) [1]	1.45 (0.92 to 8.43)	1.67 (0.90 to 8.55)	2.16 (0.90 to 8.70)
AUC_{last} (ng·h/mL)	7766 ± 2506	23086 ± 5971	52848 ± 10100
$AUC_{0-\infty}$ (ng·h/mL)	11756 ± 2405	39155 ± 9446	66747 ± 24731
$AUC_{0-\infty}$/Dose (kg·h/L)	1.1756 ± 0.2405	1.5662 ± 0.3778	1.3349 ± 0.4946
$t_{1/2,z}$ (h)	15.14 ± 3.36	30.88 ± 14.72	24.19 ± 19.21
CL/F (mL/h/kg)	879 ± 173	679 ± 197	844 ± 329
V_z/F (mL/kg)	19653 ± 6847	27189 ± 8436	24406 ± 9688

Abbreviations: t_{lag}, lag time; C_{max}, maximum plasma levofloxacin concentration in the 1st dose interval; t_{max}, time of occurrence for the maximum plasma levofloxacin concentration in the 1st dose interval; AUC_{last}, area under the plasma levofloxacin concentration-time curve from time 0 to the last measurable concentration in the 1st dose interval; $AUC_{0-\infty}$, area under the plasma levofloxacin concentration-time curve from time 0 to infinite time; $t_{1/2,z}$, terminal elimination half-life; CL/F, apparent clearance; V_z/F apparent volume of distribution in the terminal phase.

[1] Median and range are presented.

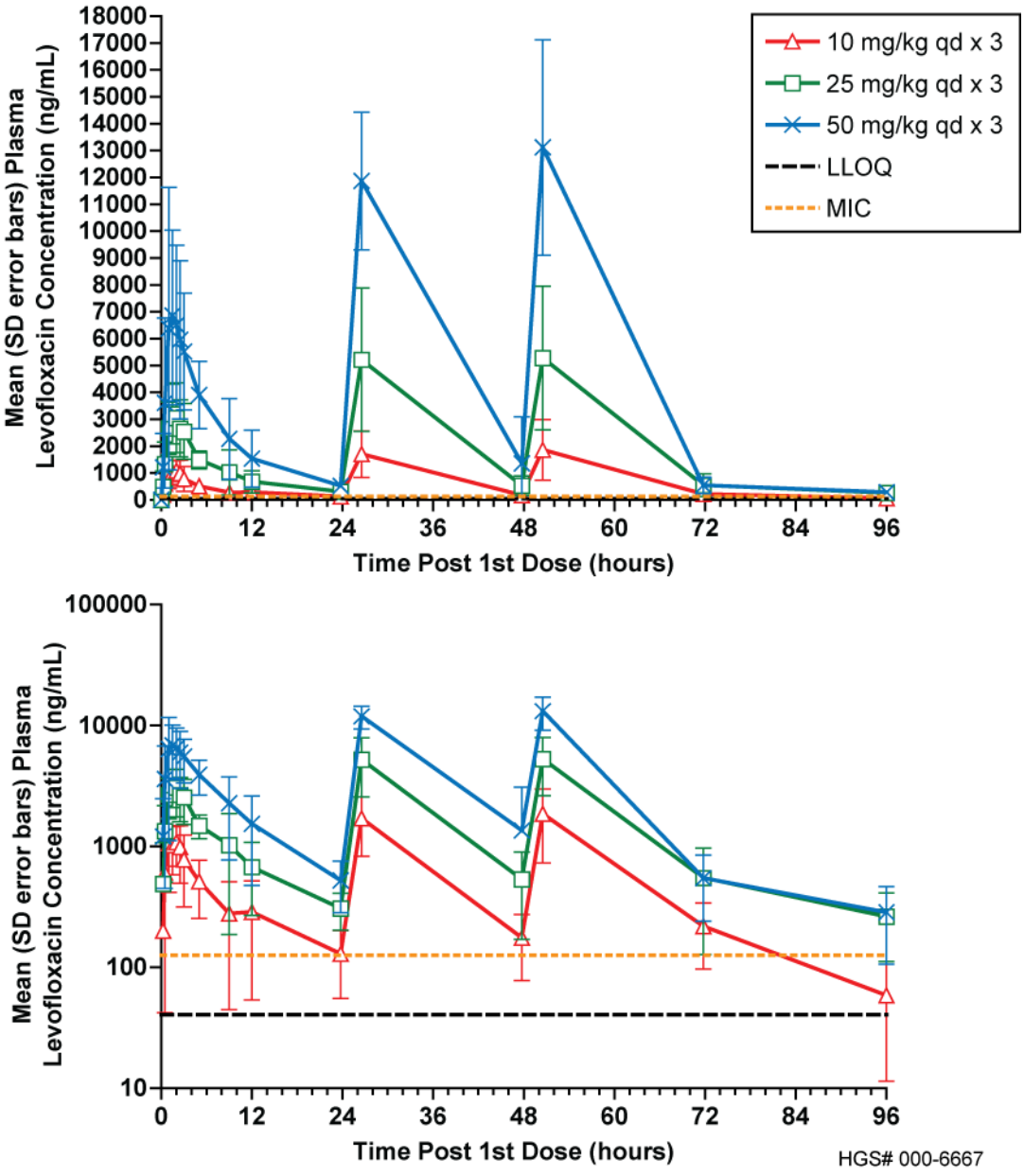

Figure 13-50 Levofloxacin plasma concentration-time profiles for rabbits administered PO 10, 25, and 50 mg/kg levofloxacin (mean ± SD) (723-G005835)

Upper panel, linear scale; lower panel, semilog scale. The minimum inhibitory concentration (MIC) of levofloxacin against Ames strain *B. anthracis* reported in the Levaquin product label (2007) is shown for comparison. The LLOQ for levofloxacin is 40.53 ng/mL.

13.15.3.2 Raxibacumab and Levofloxacin Combination Study (Study 781-G923701) (AB50409.INF.0.043)

The PK of raxibacumab and levofloxacin (Report AB50409.INF.0.043) were evaluated in rabbits symptomatic for inhalation anthrax as part of the GLP study to evaluate the efficacy of raxibacumab in combination with levofloxacin (Study 781-G923701).

In total, 52 NZW rabbits were randomized by sex and weight into 2 treatment groups of 20 animals each (10 male, 10 female) and 1 control group of 12 animals (6 male, 6 female). All rabbits were aerosol challenged on Day 0 with a targeted 200 x LD_{50} dose of *B. anthracis* (Ames strain) spores. Following detection of serum PA or body temperature increase, rabbits were treated IG with 50 mg/kg levofloxacin or placebo (sterile water for injection), which was followed by a single bolus IV injection of 40 mg/kg raxibacumab) or vehicle. Levofloxacin (or placebo) was administered once daily for 3 days.

Blood samples for the levofloxacin assay were collected from all rabbits at 7 days prior to spore challenge and just prior to the 1st dose; at 2 hours after administration of each dose; at 24 hours after each dose (just prior to the subsequent dose); and at 48 hours after the 3rd dose. Blood samples for the raxibacumab assay were collected from all rabbits at 7 days prior to spore challenge; just prior to levofloxacin, raxibacumab, or vehicle dosing; at 5 minutes after dosing; at 8, 24, 48, and 96 hours after dosing; and at 7, 14, 21, and 28 days after spore challenge. When feasible, a terminal blood sample was taken just prior to euthanasia for animals that were judged to be moribund. Plasma samples were analyzed for levofloxacin using a high performance liquid chromatography/mass spectroscopy/mass spectroscopy assay. The calibration range for the assay was from 40.40 to 16159.00 ng/mL. Plasma samples were analyzed for raxibacumab using an ECL-based assay.

Plasma levofloxacin concentration-time profiles for all rabbits were analyzed individually. The maximum plasma levofloxacin concentration after the nth dose, defined as the concentration measured 2 hour after the dose ($C_{max,n}$) and minimum plasma levofloxacin concentration after the nth dose, defined as the concentration measured just prior to the subsequent dose ($C_{min,n}$) results are shown in Table 13-44. Unpaired t-tests of $C_{max,n}$ and $C_{min,n}$ between the levofloxacin alone and levofloxacin/raxibacumab combination groups show no differences that could be attributed to altered levofloxacin PK after raxibacumab administration.

Table 13-44 $C_{max,n}$ and $C_{min,n}$ in rabbits administered levofloxacin +/- raxibacumab (781-G923701)

	Group 2 Levofloxacin Alone		Group 3 Levofloxacin Plus Raxibacumab		
	N	Mean ± SD	N	Mean ± SD	P-Value[1]
$C_{max,1}$ (ng/mL)	20	4148.6 ± 899.6	20	3907.5 ± 817.8	0.3808
$C_{min,1}$ (ng/mL)	20	176.4 ± 86.4	20	165.0 ± 76.1	0.6599
$C_{max,2}$ (ng/mL)	20	4983.2 ± 1402.3	19	4583.7 ± 1581.0	0.4086
$C_{min,2}$ (ng/mL)	20	199.5 ± 125.8	18	165.2 ± 86.3	0.3396
$C_{max,3}$ (ng/mL)	20	4470.2 ± 913.7	18	3934.9 ± 1200.9	0.1285
$C_{min,3}$ (ng/mL)	20	197.5 ± 137.0	19	195.7 ± 124.7	0.9667

[1] Abbreviations: $C_{max,n}$, maximum plasma levofloxacin concentration after the n^{th} dose, defined as the concentration measured 2 hour after the dose; $C_{min,n}$, minimum plasma levofloxacin concentration after the n^{th} dose, defined as the concentration measured just prior to the subsequent dose, or at 24 hours after the 3rd dose; NA, not applicable.

[2] From an unpaired t-test.

PK analyses of raxibacumab concentration-time profiles were conducted using population analysis techniques (mixed effect modeling). The plasma raxibacumab concentrations best fit a 2-compartment open model with 1st-order elimination from the central compartment. Inter-individual variability for CL, V_1, and V_2 were modeled using exponential terms. Inter-individual variability for CLD_2 was not modeled. A biphasic apparent elimination profile was noted for several rabbits, with apparent accelerated elimination in the latter portion of the profile. Immunogenicity results indicated 18 out of 19 rabbits included in the analysis had anti-raxibacumab antibody titers that were positive in the immunogenicity screening assay on Day 14 post-challenge. Immunogenicity outcome was found to be a significant covariate for CL. Body weight was found to be a significant covariate for V_1. Other factors assessed (sex, age, size of spore challenge, duration of spore challenge, time to 1st bacteremia by culture, and bacteremia outcome at each collection time) were not significant covariates accounting for inter-individual differences in PK. Survival time and survival status were not assessed as potential covariates due to the high survival rate for rabbits treated with levofloxacin plus raxibacumab. The parameter estimates for the PK model are summarized in Table 13-45. Raxibacumab PK from this study (as administered in combination with levofloxacin) was consistent with the PK results of raxibacumab administered alone in the pivotal rabbit efficacy.

Table 13-45 Raxibacumab PK parameters in rabbits levofloxacin + raxibacumab (781-G923701)

Primary Parameters	Mean	CV%
V_1 (mL)	150	13.3
Effect of weight (WT) on V_1 (mL)	150+((WT-3.48)/3.48)x202	
At 2.97 kg	120	
At 3.48 kg	150	
At 4.02 kg	181	
CL (mL/day), anti-raxibacumab antibody negative	31.92	23.4
CL (mL/day), anti-raxibacumab antibody positive	64.08	
V_2 (mL)	55.3	29.1
CLD_2 (mL/day)	12.6	NA
Residual Variability, CV(%)	9.6% CV% for proportional error component 20.2 SD for additive error component	
Secondary Parameters		
C_{max} (µg/mL)[1]	928	
$AUC_{0-\infty}$ (µg·day/mL)[1]	4361	
$t_{1/2,\alpha}$ (days)[1]	0.09	
$t_{1/2,\beta}$ (days)[1]	4.49	
MRT (days)[1]	6.43	
V_{ss} (mL/kg)[1]	58.99	

Abbreviations: CV%, coefficient of variation; V_1, volume of distribution for the central compartment; CL, clearance; V_2, volume of distribution for the peripheral compartment; CLD_2, intercompartmental clearance; NA, not applicable; C_{max}, maximum plasma drug concentration; $AUC_{0-\infty}$, area under the plasma drug concentration-time curve from time 0 to infinite time; $t_{1/2,\alpha}$, elimination half-life for the 1st phase; $t_{1/2,\beta}$, elimination half-life for the 2nd (terminal) phase; MRT, mean residence time; V_{ss}, volume of distribution at steady-state.

[1] Assuming a typical anti-raxibacumab antibody-negative rabbit weighing 3.48 kg, and a dose of 40 mg/kg.

13.15.3.3 Raxibacumab and Ciprofloxacin in Inhalation Anthrax-Spore Challenged Monkeys (Study 789-G923702) (AB50409.INF.0.042)

The PK of raxibacumab and ciprofloxacin administered in combination (Report AB50409.INF.0.042) were evaluated in monkeys with inhalation anthrax (Study 789-G923702).

In total, 40 cynomolgus monkeys were randomized by sex into 2 treatment groups of 14 animals each (7 male, 7 female) and 1 control group of 12 animals (6 male, 6 female). On Day 0, monkeys were aerosol challenged with a targeted 200 x LD_{50} inhaled dose of *B. anthracis* (Ames strain) spores. Following detection of serum PA, monkeys were treated with 1 mg/kg diphenhydramine IM (to simulate the recommended premedication used in humans), followed within 5 minutes by 75 mg ciprofloxacin or placebo (sterile water for injection) IG, which was in turn followed by a single bolus IV injection of 40 mg/kg

raxibacumab or vehicle. Ciprofloxacin (or placebo) was administered every 12 hours (q12h) for a total of 6 doses.

Blood samples were collected for ciprofloxacin analysis at 7 days prior to spore challenge; at 1.5 hours after administration of the 1st dose; and at 12 hours after each dose (just prior to the subsequent dose). Blood samples were collected for raxibacumab analysis at 7 days prior to spore challenge; just prior to ciprofloxacin, raxibacumab, or vehicle dosing; at 5 minutes after dosing; at 24, 48, 72, and 120 hours after dosing; and at 8, 14, 21, and 28 days after spore challenge. When feasible, a terminal blood sample was taken just prior to euthanasia for animals that were judged to be moribund. Serum samples were analyzed for ciprofloxacin using a HPLC/mass spectroscopy/mass spectroscopy assay. The calibration range for the assay was from 10 to 5000 ng/mL. Serum samples were analyzed for raxibacumab using an ECL-based assay.

Serum ciprofloxacin concentration-time profiles for all monkeys were analyzed individually. The mean $C_{max,1}$ and $C_{min,n}$ results are summarized in Table 13-46. Unpaired t-tests of $C_{max,n}$ and $C_{min,n}$ between the ciprofloxacin alone and ciprofloxacin/raxibacumab combination groups show no differences that could be attributed to altered ciprofloxacin PK after raxibacumab administration.

Table 13-46 $C_{max,1}$ and $C_{min,n}$ in monkeys administered ciprofloxacin +/- raxibacumab (789-G923702)

	Group 2 Ciprofloxacin Alone		Group 3 Ciprofloxacin plus Raxibacumab		
	N	Mean ± SD	N	Mean ± SD	P-value[1]
$C_{max,1}$ (ng/mL)	14	675.3 ± 476.5	14	1119.3 ± 912.2	0.1225
$C_{min,1}$ (ng/mL)	14	193.1 ± 117.1	14	194.5 ± 75.8	0.9699
$C_{min,2}$ (ng/mL)	14	257.1 ± 118.0	14	238.0 ± 130.9	0.6879
$C_{min,3}$ (ng/mL)	14	335.4 ± 153.4	13	229.6 ± 119.9	0.0580
$C_{min,4}$ (ng/mL)	14	264.9 ± 120.6	13	244.2 ± 132.2	0.6743
$C_{min,5}$ (ng/mL)	14	266.2 ± 144.6	13	230.7 ± 111.9	0.4846
$C_{min,6}$ (ng/mL)	14	243.0 ± 141.6	13	201.1 ± 99.5	0.3858

Abbreviations: $C_{max,n}$, maximum plasma ciprofloxacin concentration after the n^{th} dose, defined as the concentration measured 1.5 hour after the dose; $C_{min,n}$, minimum plasma ciprofloxacin concentration after the n^{th} dose, defined as the concentration measured just prior to the subsequent dose; NA, not applicable.

[1] From an unpaired t-test.

PK analyses of raxibacumab concentration-time profiles were conducted using population analysis techniques (mixed effect modeling). The serum raxibacumab concentrations best fit a 2-compartment open model with 1st-order elimination from the central compartment. Inter-individual variability for CL, V_1, and V_2 were modeled using exponential terms. Inter-individual variability for CLD_2 was not modeled. Body weight was found to be a significant covariate for CL, V_1, and V_2. Other factors assessed (sex, age, duration of spore challenge, size of spore challenge, and bacteremia status at each collection time, and

time to 1st bacteremia by culture) were not significant covariates accounting for inter-individual differences in PK. The parameter estimates for the PK model are summarized in Table 13-47. Overall, these results indicate that there is minimal variability in raxibacumab disposition, even in monkeys exhibiting symptoms of inhalation anthrax.

These results can be compared with those for the 40 mg/kg dose in the raxibacumab monotherapy study, Study 724-G005829 (Report AB50409.INF.0.040). The raxibacumab alone study, C_{max} were 502 and 1004 µg/mL and $AUC_{0-\infty}$ were 2966 and 5931 µg·day/mL for male and female animals, respectively, whereas in Study 789-G923702, C_{max} was 1060 µg/mL and $AUC_{0-\infty}$ was 10782 µg·day/mL. In the monotherapy study, CL was 20 mL/day, while $t_{1/2,\beta}$ were 10 and 9 days, and $t_{1/2,\alpha}$ were 0.5 and 0.8 days for male and female animals, respectively, while in the combination study CL was 11 mL/day, $t_{1/2,\beta}$ was 16 days, and $t_{1/2,\alpha}$ was 0.6 days. In the 724-G005829 study, V_1 was 160 mL, while in the combination study V_1 was 117 mL. V_2 was similar between the 2 studies, at 125 mL and 139 mL for the prior and current studies, respectively. CL was slower in healthy animals. The rapid resolution of bacteremia by ciprofloxacin in the combination study could alter the anthrax disease state to resemble more closely that of a healthy animal. Hence, it seems reasonable to expect that in the combination study, raxibacumab PK would be more similar to those of healthy animals. On this basis, it was judged that raxibacumab PK were unaffected by co-administration of ciprofloxacin in the current study, as was the case for the human clinical trial of raxibacumab administered in combination with ciprofloxacin in healthy subjects.

Table 13-47 Raxibacumab PK parameters in monkeys administered ciprofloxacin +/- raxibacumab (789-G923702)

Primary Parameters	Mean	CV%
V_1 (mL)	117	13.6
Effect of weight on V_1 (mL)	117 + 80.0([weight – 3.1]/3.1)	
At 2.4 kg	99	
At 3.1 kg	117	
At 6.4 kg	202	
CL (mL/day)	11.472	23.0
Effect of weight on CL (mL/day)	11.472 + 72.24([weight – 3.1]/3.1)	
At 2.4 kg	15.155	
At 3.1 kg	11.472	
At 6.4 kg	93.334	
V_2 (mL)	139	17.3
Effect of weight on V_2 (mL)	139 + 225([weight – 3.1]/3.1)	
At 2.4 kg	88	
At 3.1 kg	139	
At 6.4 kg	379	
CLD_2 (mL/day)	302	NA
Residual Variability, CV(%)	6.59	
Secondary Parameters		
C_{max} (µg/mL)[1]	1060	
$AUC_{0-\infty}$ (µg·day/mL)[1]	10782	
$t_{1/2,\alpha}$ (days)	0.64	
$t_{1/2,\beta}$ (days)	16.27	
MRT (days)	22.26	
V_{ss} (mL)[1]	256	

Abbreviations: CV%, coefficient of variation; V_1, volume of distribution for the central compartment; CL, clearance; V_2, volume of distribution for the peripheral compartment; CLD_2, intercompartmental clearance; NA, not applicable; C_{max}, maximum serum drug concentration; $AUC_{0-\infty}$, area under the serum drug concentration-time curve from time 0 to infinite time; $t_{1/2,\alpha}$, elimination half-life for the 1st phase; $t_{1/2,\beta}$, elimination half-life for the 2nd (terminal) phase; MRT, mean residence time; V_{ss}, volume of distribution at steady-state.

[1] Assuming a typical monkey weighing 3.1 kg and a dose of 40 mg/kg.

13.15.3.4 Raxibacumab and Ciprofloxacin in Healthy Human Volunteers (HGS1021-C1064)

HGS1021-C1064 was an open-label study to evaluate the safety and PK of raxibacumab administered alone or in combination with ciprofloxacin in healthy adult male and female subjects. Three treatment groups were evaluated:

Group 1: Subjects received 15 doses of PO ciprofloxacin (500 mg q12h) from Day 0 to Day 7. On Day 5, prior to the 1st daily ciprofloxacin dose, the subjects also received a single dose of raxibacumab (40 mg/kg) IV.

The dosage schedule of raxibacumab on Day 5 of a 7-day dosing period of PO ciprofloxacin allowed for assessment of the impact of raxibacumab on steady state ciprofloxacin levels. Also, this dosage schedule was consistent with a clinical scenario in which PO ciprofloxacin is prescribed for suspected anthrax exposure, with IV raxibacumab administered subsequently as clinically indicated for confirmed disease or onset of clinical symptoms.

Group 2: Subjects received a single dose of raxibacumab (40 mg/kg) IV on Day 0.

This group constitutes the comparator group for safety and PK of raxibacumab administered alone.

Group 3: Subjects received a dose of IV ciprofloxacin 400 mg on Day 0 immediately followed by IV raxibacumab 40 mg/kg. A 2nd dose of IV ciprofloxacin 400 mg was administered on Day 0 12 hours after the 1st infusion was completed. Subjects in Group 3 continued with PO ciprofloxacin 500 mg every 12 hours on Days 1-7 for a total of 13 doses.

This dosage schedule of concomitant administration of IV ciprofloxacin with IV raxibacumab is consistent with the anticipated use of raxibacumab in the event of symptomatic anthrax infection.

Subjects were 1st randomized in a 1:1 ratio to Group 1 or Group 2. When enrollment of Group 1 and Group 2 was completed, subjects were enrolled into Group 3. In total, 88 subjects were randomized and treated (32 in Group 1, 28 in Group 2, and 28 in Group 3) in this study. There were a similar number of male and female subjects in this study (43 males and 45 females). The majority of the subjects in this study were white (53/88, 60%), with an age range of 18 to 60 years and weight range of 49 to 99 kg. In total, 86 subjects were considered evaluable for raxibacumab PK and 56 subjects were evaluable for ciprofloxacin PK. The PK parameters for raxibacumab and ciprofloxacin were calculated using noncompartmental techniques.

Blood samples for plasma ciprofloxacin concentration measurement were collected from Group 1 and Group 3 subjects at selected timepoints throughout the study. Plasma samples were analyzed for ciprofloxacin using a HPLC/mass spectrometry method. The assay was validated over a calibration range of 10 ng/mL to 5000 ng/mL in human plasma.

Blood samples for serum raxibacumab concentration measurement were to be collected from all subjects just prior to administration of the raxibacumab dose, at 5 minutes and 8 hours after completion of the raxibacumab infusion, and at 1, 3, 7, 14, 21, 28, 42, and 56 days after the raxibacumab dose. Serum samples were analyzed for raxibacumab using the same ECL-based assay as used in both studies HGS1021-C1063 and HGS1021-C1069.

Plasma ciprofloxacin concentration-time profiles were very similar for Group 1 prior to raxibacumab dosing (Dose 9) and following treatment with raxibacumab (Dose 11), with overlapping SD error bars (Figure 13-51). The 90% CI for the primary PK parameters $C_{ss,max}$ and AUC_{τ} were within the 80% to 125% equivalence limits. Hence, it is concluded that ciprofloxacin exposure was equivalent when ciprofloxacin was administered alone or in combination with raxibacumab.

Table 13-48 Ciprofloxacin PK parameters with and without co-administration with raxibacumab (HGS1021-C1064)

	Group 1		Group 3	
Parameter	**PO, Without Raxibacumab (n = 30)**	**PO, With Raxibacumab (n = 30)**	**IV, With Raxibacumab (n = 28)**	**PO, With Raxibacumab (n = 28)**
C_{max} (ng/mL)	NA	NA	1854 ± 402	NA
$C_{ss,max}$ (ng/mL)	1436 ± 519	1419 ± 599	NA	1195 ± 566
t_{max} (h)	NA	NA	1.32 (1.23 to 1.60)[1]	NA
$t_{ss,max}$ (h)	1.75 (0.50 to 4.00)[1]	2.00 (0.58 to 4.12)[1]	NA	1.00 (0.5 to 6.00)[1]
$AUC_{0-\infty}$ (ng·h/mL)	NA	NA	8770 ± 1877	NA
AUC_{0-8h} (ng·h/mL)	6413 ± 2241	6742 ± 2279	NA	NA
AUC_{T} (ng·h/mL)	7694 ± 2680	8151 ± 2673[2]	NA	6615 ± 3224
F (%)	NA	NA	NA	58.99 ± 21.25
$t_{1/2,z}$ (h)	4.74 ± 2.09	5.25 ± 2.47	4.53 ± 0.89	4.62 ± 1.07
MRT (h)	NA	NA	6.01 ± 0.86	NA
CL or CL/F (mL/h)	72422 ± 23985	66781 ± 21874	47555 ± 9847	91061 ± 36852
V_{ss} (mL)	NA	NA	285928 ± 72589	NA
V_z or V_z/F(mL)	510699 ± 340305	486047 ± 212832	312015 ± 93369	630702 ± 308589

Abbreviations: C_{max}, maximum plasma drug concentration for a single dose; $C_{ss,max}$, maximum plasma drug concentration during a steady-state dosing interval; t_{max}, time of occurrence for C_{max}; $t_{ss,max}$, time of occurrence for $C_{ss,max}$; AUC_{0-8h}, area under the plasma drug concentration-time curve from 0 to 8 h post dose; $AUC_{0-\infty}$, area under the plasma drug concentration-time curve from time zero to infinite time for a single dose; AUC_{T}, area under the plasma drug concentration-time curve during a steady-state dosing interval; F, bioavailable fraction for oral dosing; $t_{1/2,z}$, elimination half-life for the terminal phase; MRT, mean residence time; CL, clearance; CL/F, apparent clearance for oral dosing; V_{ss}, volume of distribution at steady-state; V_z, volume of distribution in the terminal phase; V_z/F, apparent volume of distribution in the terminal phase for oral dosing; NA, not applicable.

[1] Median and range are reported.

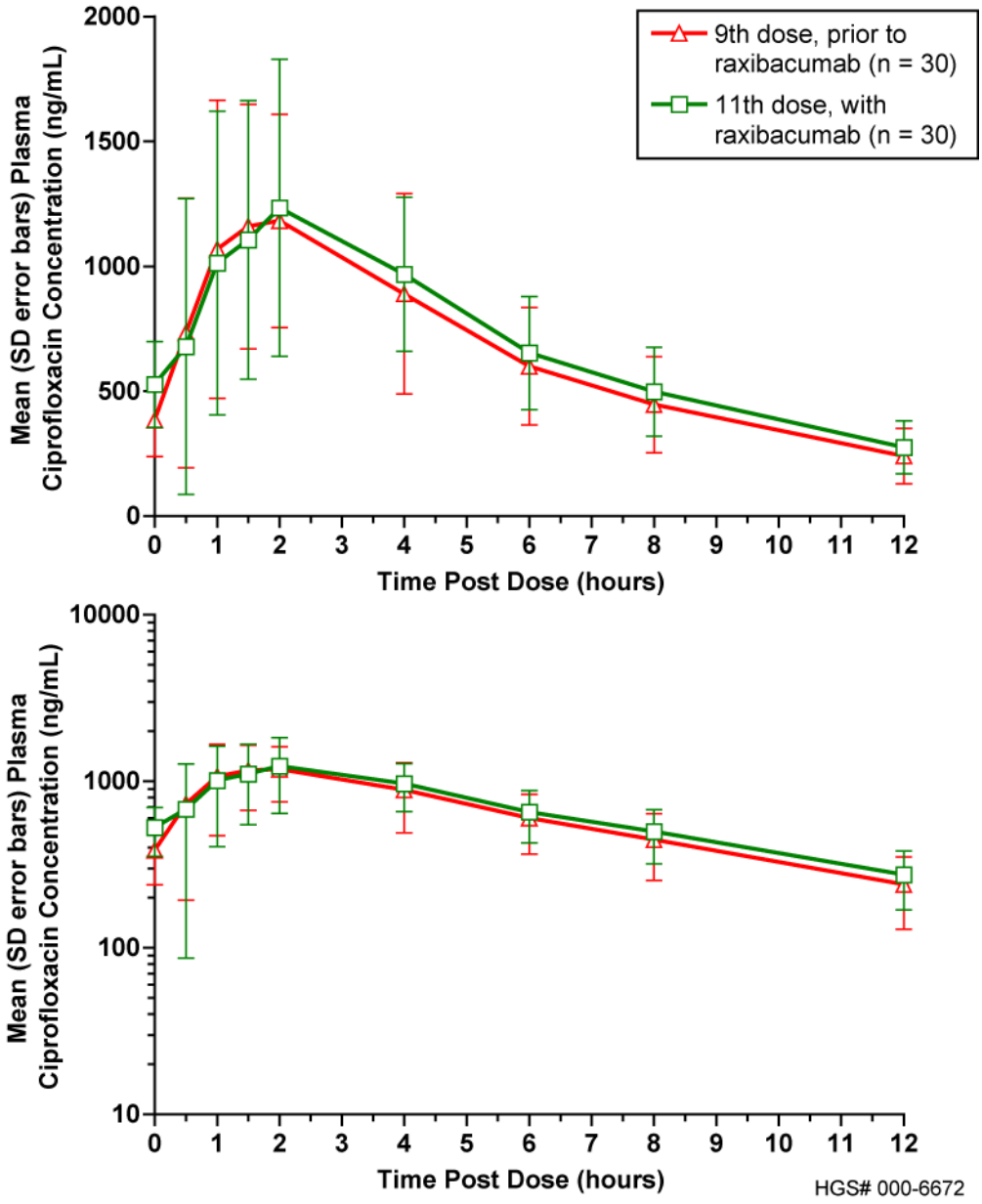

Figure 13-51 Ciprofloxacin plasma concentrations +/- raxibacumab (mean ± SD) (HGS1021-C1064)

Top panel – linear plot; bottom panel – semi log plot.

For raxibacumab, serum concentrations are shown in Figure 13-52 and noncompartmental PK results are summarized in Table 13-49, including comparison of raxibacumab administered alone or in combination with ciprofloxacin. Overall, raxibacumab PK were not affected by co-administration of IV or PO ciprofloxacin.

Table 13-49 Raxibacumab PK parameters +/- ciprofloxacin (mean ± SD) (HGS1021-C1064)

Parameter	Group 1 Raxibacumab with PO Ciprofloxacin (n = 29)	Group 2 Raxibacumab Alone (n = 28)	Group 3 Raxibacumab with IV & PO Ciprofloxacin (n = 28)
C_{max} (µg/mL)	1103 ± 225	988 ± 220	1048 ± 180
C_{max}/Dose (kg/mL)	0.0287 ± 0.0042	0.0251 ± 0.0040	0.0262 ± 0.0045
	(p = 0.0015[1])		(p = 0.3310[1])
t_{max} (day)[2]	0.098 (0.049 to 0.430)	0.103 (0.038 to 0.439)	0.107 (0.099 to 0.914)
	(p = 0.0108[1])		(p = 0.1120[1])
$AUC_{0-\infty}$ (µg·day/mL)	14362 ± 4208	15328 ± 5059	16349 ± 4256
$AUC_{0-\infty}$/Dose (kg·day/mL)	0.3733 ± 0.0931	0.3873 ± 0.1145	0.4085 ± 0.1064
	(p = 0.6157[1])		(p = 0.4743[1])
$t_{1/2,z}$ (day)	19.66 ± 7.89	20.44 ± 6.46	21.50 ± 8.92
	(p = 0.6886[1])		(p = 0.6125[1])
MRT (day)	24.68 ± 7.87	27.30 ± 8.24	27.79 ± 9.69
	(p = 0.2246[1])		(p = 0.8406[1])
CL (mL/day/kg)	2.98 ± 1.33	2.85 ± 1.03	2.63 ± 0.82
	(p = 0.6865[1])		(p = 0.3904[1])
V_{ss} (mL/kg)	64.85 ± 11.60	71.74 ± 17.36	67.17 ± 12.61
	(p = 0.0825[1])		(p = 0.2646[1])
V_z (mL/kg)	74.33 ± 23.43	77.29 ± 20.50	74.27 ± 18.94
	(p = 0.6150[1])		(p = 0.5696[1])

Abbreviations: C_{max}, maximum serum raxibacumab concentration for a single dose; t_{max}, time of occurrence for C_{max}; $AUC_{0-\infty}$, area under the serum raxibacumab concentration-time curve from time 0 to infinite time for a single dose; $t_{1/2,z}$, elimination half-life for the terminal phase; MRT, mean residence time; CL, clearance; V_{ss}, volume of distribution at steady-state; V_z, volume of distribution in the terminal phase.

1 P-value from a 2-sample t-test with Group 2 (raxibacumab alone) as the reference treatment; the exception is t_{max}, for which the p-value is from a Mann-Whitney test.

2 Median and range are presented.

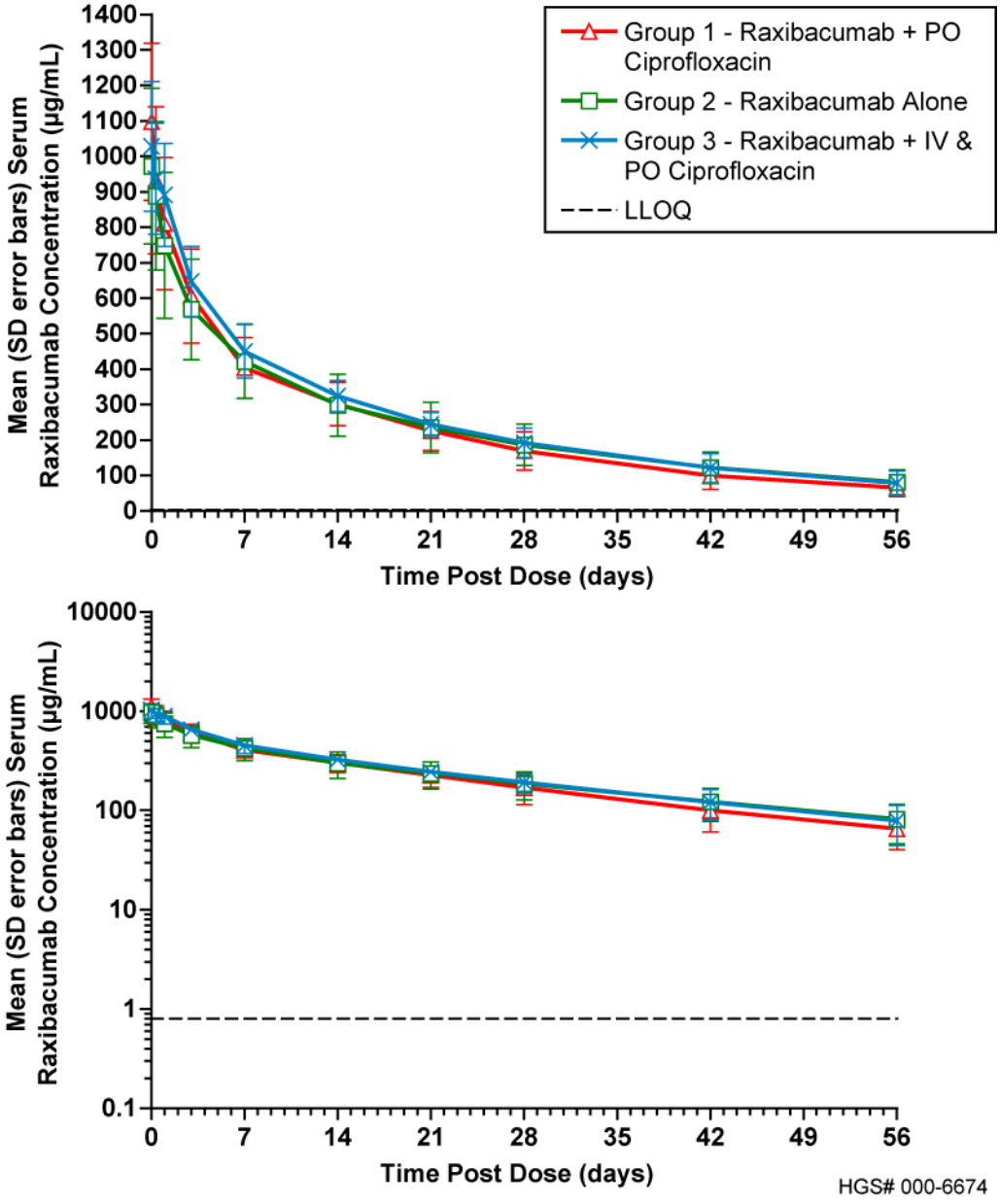

Figure 13-52 Raxibacumab serum concentrations +/- ciprofloxacin (mean ± SD) (HGS1021-C1064)

Top panel – linear plot; bottom panel – semi log plot.

13.16 Appendix 16: Protective Antigen (PA) Kinetics in Inhalation Anthrax-Spore Challenged Rabbits and Monkeys

Raxibacumab binds the PA of *B. anthracis* and prevents PA-induced toxicity by inhibiting the interaction of PA with its receptor. Because circulating PA is both the target for raxibacumab and the means of anthrax toxicity, the kinetics of PA elaboration and resolution were evaluated in the rabbit and monkey anthrax model characterization studies (615-N104504 and 685-G005762) and in the therapeutic efficacy studies (682-G005758, 724-G005829, 781-G923701 and 789-G923702). PA levels in untreated or placebo-treated animals were also used to perform population PA kinetic modeling in each of the species.

13.16.1 PA in Rabbits

13.16.1.1 Rabbit Model Characterization Study (615-N104504)

Serum PA kinetics were analyzed as part of the rabbit characterization study (Study 615-N104504).

Individual serum PA concentration-time profiles were generated and, in general, the serum PA concentration-time profiles comprised 3 phases: an initial rapid rise, followed by a plateau period, and a terminal phase during which levels increased rapidly again. The mean serum PA concentration-time profile shows an initial rise up to about 36 h post challenge, followed by a period during which the concentrations plateau, followed by a 2nd period of rising concentrations (Figure 13-53). These observations are consistent with a disease progression in which anthrax spores vegetate and begin to produce PA (the initial rise in the profile), followed by a period in which PA production is slowed, or during which PA clearance increases (the plateau), followed by increased PA production/slowed PA clearance (the 2nd rise).

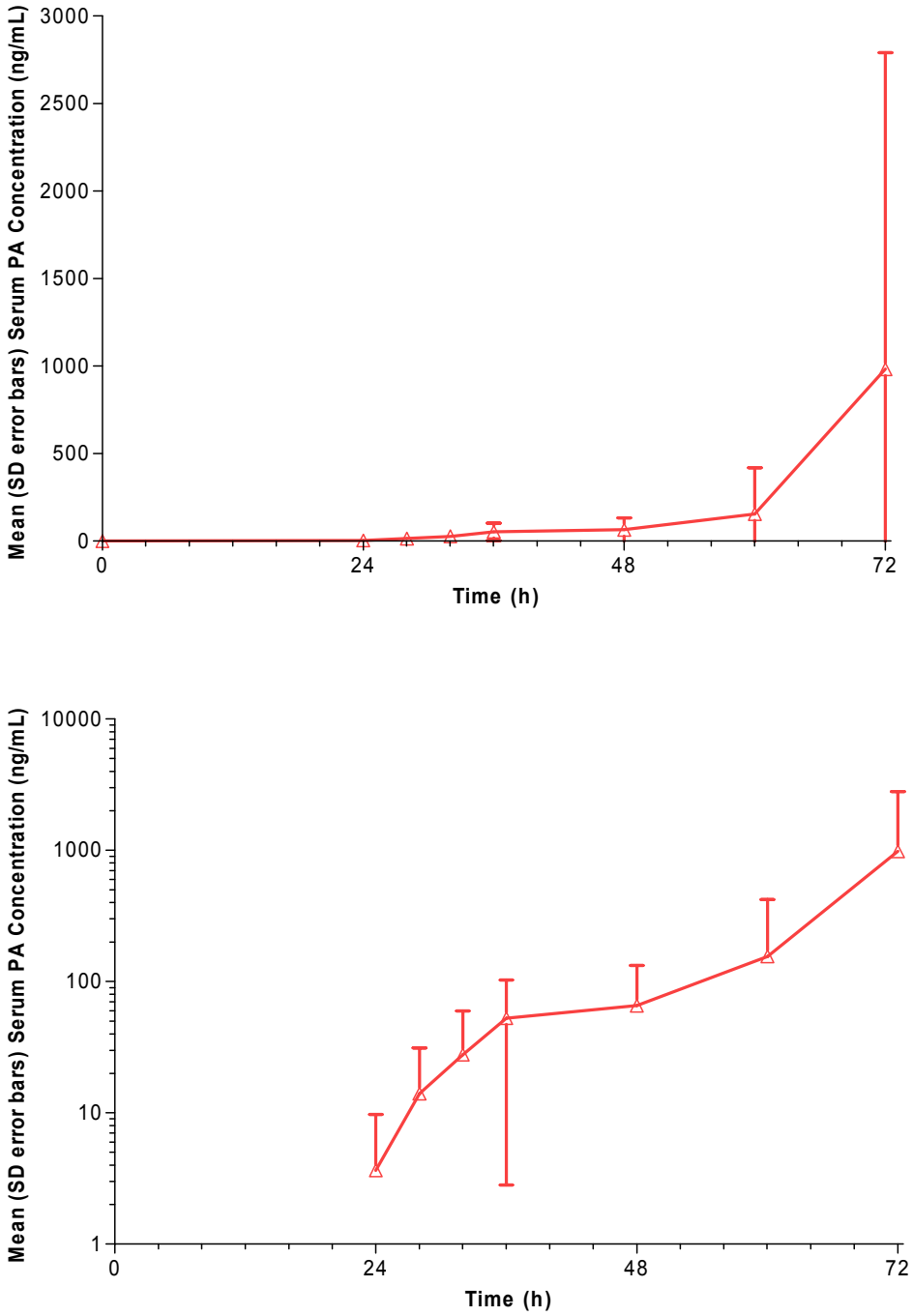

Figure 13-53 Serum PA concentration-time profile in rabbits (615-N104504)

(upper panel – linear plot; lower panel – semi-log plot)

The PA results from this study were used in the population PA kinetic analysis.

The times to events in the serum PA concentration-time profile (t_{lag}, t_{1st}, t_{max}, $t_{plateau}$, and $t_{2ndrise}$) were compared with the times to relevant clinical observations (survival time, time to 1st detected bacteremia, time to confirmed bacteremia by polymerase chain reaction (PCR), and time to 1st significant increase in body temperature). These comparisons are summarized in Table 13-50. It should be noted that t_{1st} either coincides with the time to 1st detected bacteremia or occurred 4 hours later, which indicates that the appearance of measurable PA in serum is associated with bacteremia, either by culture or by PCR, as expected. In addition, t_{1st} occurred 3 h earlier, on average, than the 1st significant temperature elevation (range: 1 to 5 h). This suggests that as might be expected, the appearance of PA in serum is nearly coincident with the appearance of bacteremia, and is an antecedent to increased temperature. Based on the results presented in Table 13-50, other serum PA kinetic parameters appear to be less closely associated with the clinical observations.

The onset of clinical observations relevant to the disease generally correlate well with parameters describing the times to events in the serum PA concentration-time profile. Values of t_{1st} either coincides with the time to 1st detected bacteremia or occurred 4 h later, which indicates that the appearance of measurable PA in serum is associated with the appearance of bacteremia, as expected. Due to the high multiples of LD_{50} used in the spore challenge, which likely leads to the presence of serum PA concentrations that were in excess of those needed to produce disease worsening to death, it was not possible to detect correlations between disease relevant clinical observations and parameters reflecting the magnitude of systemic PA exposure.

Table 13-50 Serum PA concentration-time curve kinetic parameters and relevant clinical observations in rabbits (615-N104504)

	Time of Clinical Observation (h)	Elapsed Time Between Clinical Observation and Serum PA Concentration-Time Profile Events (h): t_{lag}	t_{1st}	t_{max}	$t_{plateau}$	$t_{2ndrise}$
N	**7**	**7**	**7**	**7**	**7**	**6**
	Survival Time					
Mean	85.1	69.1	56.5	18.2	48.0	35.2
Min	48.5	48.5	24.5	0.5	12.5	17.9
Median	77.9	69.1	48.8	9.1	41.9	29.6
Max	116.6	92.4	88.4	44.6	80.4	56.6
	Time to 1st Detected Bacteremia by Culture					
Mean	27	11	-2	-40	-10	-28
Min	20	0	-4	-48	-16	-36
Median	24	4	0	-40	-12	-26
Max	36	24	0	-28	-4	-24

Table 13-50 Serum PA concentration-time curve kinetic parameters and relevant clinical observations in rabbits (615-N104504)

	Time of Clinical Observation (h)	Elapsed Time Between Clinical Observation and Serum PA Concentration-Time Profile Events (h): t_{lag}	t_{1st}	t_{max}	$t_{plateau}$	$t_{2ndrise}$
N	7	7	7	7	7	6
	Time to Confirmed Bacteremia by PCR					
Mean	27	11	-1	-39	-10	-27
Min	20	0	-4	-48	-16	-32
Median	28	4	0	-40	-8	-26
Max	36	24	0	-28	-4	-24
	Time of 1st Significant Temperature Increase					
Mean	31.6	15.6	3.1	-35.2	-5.5	-23.6
Min	27.0	5.3	1.3	-45.0	-8.8	-29.4
Median	30.6	8.8	3.0	-37.0	-5.4	-23.0
Max	40.8	27.6	4.8	-20.8	-1.0	-19.2

Abbreviations: t_{lag}, the time of the last serum PA concentration that was less than the lower limit of quantitation; t_{1st}, time to C_{1st}; t_{max}, time to C_{max}; $t_{plateau}$, time which marks the beginning of the plateau portion of the serum PA concentration-time profile; $t_{2nd\ rise}$, time at which the 2nd increasing phase of the profile began.

(concluded)

13.16.1.2 Rabbit Therapeutic Efficacy Study (Study 682-G005758) (AB50409.INF.0.036)

Serum PA kinetics were also analyzed as part of the rabbit pivotal efficacy study (Study 682-G005758). Serum samples were analyzed for total PA (free + raxibacumab-bound) concentrations using an ECL-based bridging assay. The LLOQ was 0.34 ng/mL of PA in 100% rabbit serum and the lower limit of detection (LLOD) was 0.228 ng/mL of PA in 100% rabbit serum. The upper limit of quantitation is 410000 ng/mL of PA in 100% rabbit serum.

Blood specimens for serum PA were collected 3 days prior to spore challenge and at 12, 16, 20, 24, 28, 32, and 36 hours post challenge, unless treatment occurred prior to the collection time. Additional blood specimens were collected just prior to dosing; and at 5 minutes as well as at 4, 10, 24, 36, 48, 72, 144, and 216 hours after dosing. When feasible, a terminal blood sample was to be taken from any animal found dead or just prior to euthanasia.

Examination of the profiles for the individual animals that died in all treatment groups revealed serum PA concentration-time profiles generally followed the pattern of rise-plateau-rise, with the exception that some animals died early enough that the 2nd rising phase was not observed (Figure 13-54). For the animals that survived in the 20 and 40 mg/kg raxibacumab groups, serum PA showed an initial rise, but before the plateau phase was attained, serum PA concentrations began to decline. Examination of the individual animals' serum PA-concentration-time profiles confirmed that this increasing then decreasing type of PA profile is generally characteristic of surviving animals, and is not observed in those

animals that died. The PA results from this study were used in the population PA kinetic analysis.

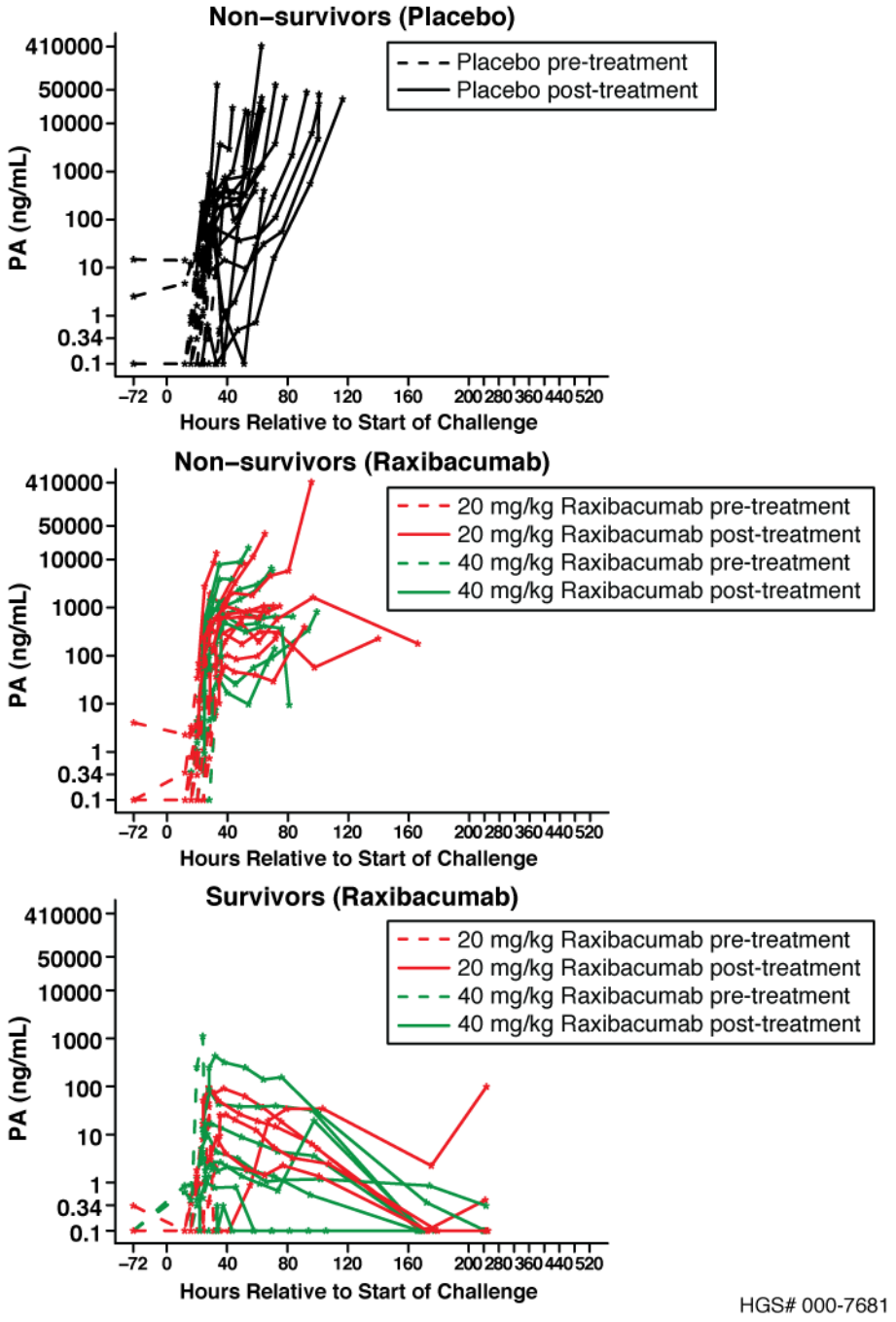

Figure 13-54 Serum PA concentration-time profiles in rabbits (682 G005758)

Review of the serum PA concentration-time results for the surviving animals, in comparison with the results of bacteremia by culture tests, revealed that the decline in serum PA concentrations coincided with the sterilization of bacteremia after raxibacumab treatment. The onset of declining serum PA concentrations was generally associated with attainment of negative bacteremia results. For all surviving animals in the 40 mg/kg dose group, serum PA concentrations declined to below the LLOQ by the end of the study and for the 20 mg/kg dose group, 3 of the 5 surviving animals attained serum PA concentrations below the LLOQ by the end of the study.

Examination of the serum PA concentration-time profiles for the surviving rabbits in this study showed that for at least some rabbits, monotonically decreasing concentrations were present at post-raxibacumab treatment bacteremia-free collection times. Therefore, PA $t_{1/2}$ was calculated for animals that had monotonically decreasing serum PA concentrations at times post raxibacumab dose, when the animal was bacteremia negative for each successive collection time (2 in the 20 mg/kg raxibacumab treatment group and 2 in the 40 mg/kg raxibacumab treatment group). The overall mean $t_{1/2}$ for serum PA was 30 hours, and ranged from 21 hours 46 hours.

13.16.1.3 Rabbit Raxibacumab/Levofloxacin Therapeutic Efficacy Study (Study 781-G923701) (AB50409.INF.0.0.043)

Plasma PA kinetics were analyzed as part of the rabbit raxibacumab/levofloxacin therapeutic efficacy study (Study 781-G923701) and reported in the PK report (AB50409.INF.0.043). PA concentrations in plasma samples were determined using an ECL-based bridging assay. The assay is similar to that used for PA measurement in rabbit serum in Study 682-G005758, except that it was qualifed for use in rabbit plasma. The LLOQ is 0.56 ng/mL of PA in 100% rabbit plasma.

Anti-PA antibody concentrations in plasma samples were determined with an ELISA. The LLOQ is 125 ng/mL of anti-PA antibody in 100% rabbit plasma.

Toxin neutralization activity (TNA) titers in plasma samples were determined using a cell killing assay. Inhibition of PA binding is correlated with increased cell viability and is used to determine the relative titer of plasma samples compared to a known positive control. The LLOQ is < 52 titer in 100% rabbit serum. The assay was also qualified for use with rabbit plasma and the results for plasma are comparable to those for serum.

All animals in the control group died, whereas 1 animal died in the levofloxacin group and 1 animal died in the levofloxacin/raxibacumab group. These animals were excluded from the analysis. As a result, the PA kinetic analysis for animals that died was based on animals in the control group.

Blood specimens for plasma PA kinetics were collected from all rabbits at 7 days prior to spore challenge as well as at 16, 20, 24, 28, 32, and 36 hours after spore challenge, unless treatment occurred prior to the collection time. Additional blood specimens were collected just prior to levofloxacin, raxibacumab, or vehicle dosing; at 5 minutes after dosing; at 8, 24,

48, and 96 hours after dosing; and at 7, 14, 21, and 28 days after spore challenge. When feasible, a terminal blood sample was taken just prior to euthanasia for animals that were judged to be moribund. Blood specimens for plasma anti-PA antibody and TNA were collected from all rabbits at 7 days prior to spore challenge as well as at 14, 21, and 28 days after spore challenge (Figure 13-55). Examination of the profiles for the individual control group animals revealed plasma PA concentration-time profiles generally followed the pattern of rise-plateau-rise, with the exception that some animals died prior to attaining either the plateau phase or the 2nd rising phase of the plasma PA concentration-time profile.

As shown in Figure 13-56, the mean plasma PA concentration-time profiles for the animals that survived in the levofloxacin and levofloxacin/raxibacumab groups showed the initial rise, but before or shortly after the plateau phase was attained, plasma PA concentrations began to decline. This increasing then decreasing type of PA profile is generally characteristic of surviving animals, and is generally not observed in those animals that died. Rising then declining serum PA concentration-time profiles were also observed in the surviving animals from the raxibacumab efficacy study (682-G005758). The PA results from this study were used in the population PA kinetic analysis.

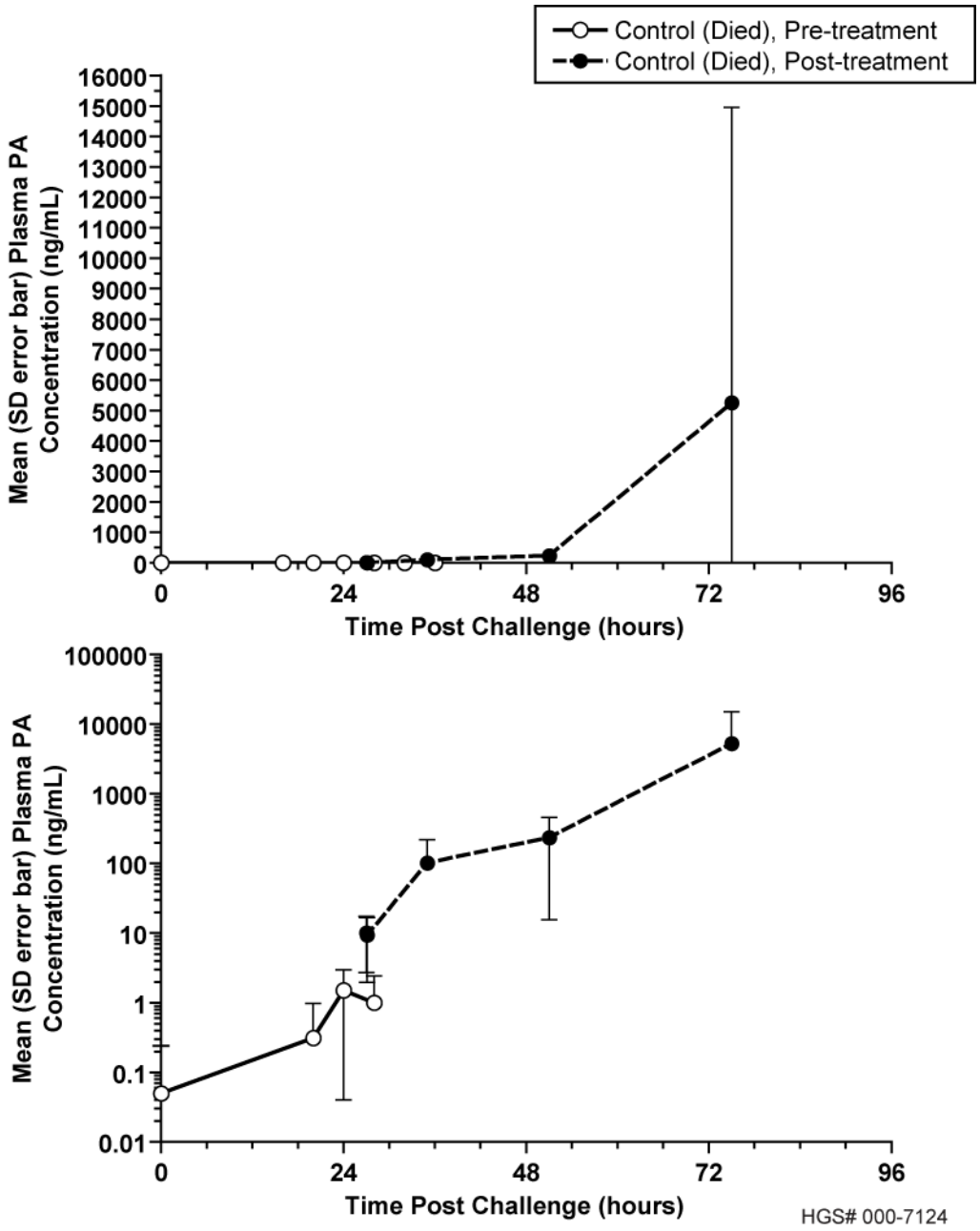

Figure 13-55 Plasma PA concentration-time profiles in rabbits that died and were administered placebo (781-G923701)

For plotting purposes, post-treatment collection times are expressed as times post challenge based on a typical treatment time of approximately 27 hours post challenge. Solid lines represent plasma PA concentrations prior to treatment intervention, while dotted lines represent plasma PA concentrations after treatment intervention.

Upper panel, linear scale; lower panel, semilog scale.

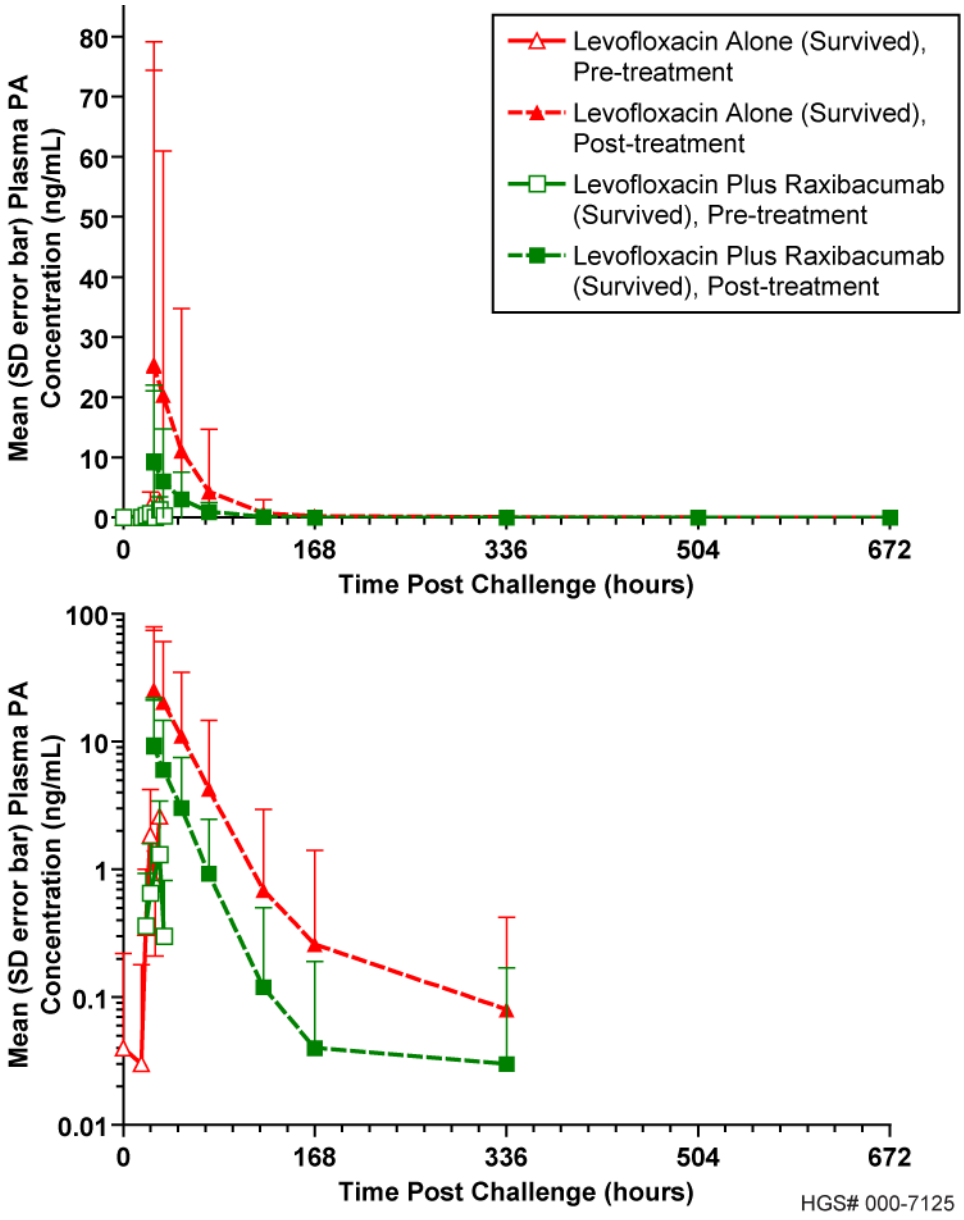

Figure 13-56 Plasma PA concentration-time profiles in rabbits that survived (781-G923701)

For plotting purposes, post-treatment collection times are expressed as times post challenge based on a typical treatment time of approximately 27 hours post challenge. Solid lines represent plasma PA concentrations prior to treatment intervention, while dotted lines represent plasma PA concentrations after treatment intervention.

Upper panel, linear scale; lower panel, semilog scale.

As noted previously, for the surviving animals' plasma PA concentrations declined after levofloxacin or levofloxacin plus raxibacumab treatment. Review of the plasma PA concentration-time results for the surviving animals, in comparison with the results of bacteremia by culture tests, revealed that the decline in plasma PA concentrations coincided with the sterilization of bacteremia after treatment.

Due to lack of material and high raxibacumab concentrations that could affect accurate measurement of anti-PA levels, only baseline and Day 28 samples were tested for anti-PA antibodies. Since 2 of the rabbits in the levofloxacin alone dose group appeared to be outliers, the analysis was also performed excluding the values for those 2 animals. The plasma anti-PA antibody concentration results for the active treatment groups, excluding the 2 outlier animals, are summarized in Table 13-51. The mean plasma anti-PA antibody concentration at 28 days post challenge for the levofloxacin alone dose group was numerically higher (48%) than that for the levofloxacin plus raxibacumab dose group, although the difference did not attain statistical significance. For the analysis which included the 2 outlier animals, the difference between dose groups was numerically larger (about 2-fold), but did not attain statistical significance. It should be noted that difference in Day 28 anti-PA antibody concentrations was not reflected in altered survival between the 2 groups, suggesting that the anti-PA antibody response in rabbits administered both levofloxacin and raxibacumab was adequate for survival.

Table 13-51 Plasma anti-PA antibody concentrations in surviving rabbits administered levofloxacin +/- raxibacumab (781-G923701)

		Plasma Anti-PA Antibody Concentration (µg/mL)	
		Pre-Challenge	28 Day Post-Challenge
Levofloxacin	N	17	17
	Mean ± SD	0.0148 ± 0.0609	78.237 ± 64.875
Levofloxacin/Raxibacumab	N	19	19
	Mean ± SD	0.000 ± 0.000	52.851 ± 40.799
	P-value[1]	0.3322	0.1643

[1] From an unpaired t-test.

13.16.2 PA in Monkeys

13.16.2.1 Monkey Model Characterization Study (685-G005762)

Serum PA concentration-time profiles for each monkey were analyzed individually in Study 685-G005762. In general, the serum PA concentration-time profiles of animals that died were comprised of 3 phases; an initial rapid rise, followed by a 'plateau' period of more slowly increasing or even decreasing levels, and a terminal phase during which levels increased rapidly again. Two monkeys survived until Day 30 and the serum PA-time profiles for those 2 monkeys differed from those for the monkeys that died. In particular, serum PA concentrations reached an initial peak and then declined to < LLOQ within 21 days post challenge for the survivors. The mean (± SD) serum PA concentration-time profile for the monkeys that died, as well as the profiles for the 2 monkeys that survived, are provided in

Figure 13-57. For the monkeys that survived, the initial rise in serum PA concentrations was delayed relative to the mean profile for the animals that died, and the peak serum PA concentration observed in the surviving animals was lower than the mean concentration-time profile for the animals that died. Due to the obvious difference in serum PA disposition between animals that survived and those that died, subsequent analyses of serum PA kinetics were based on the subgroup of animals that died.

There were 5 males and 1 female in the group of animals that died, while both surviving animals were males. The serum PA concentrations for the female monkey were within the 95% CI of those for the male monkeys. The concentrations for the female were not consistently lower or higher than the mean concentrations for the male monkeys at corresponding collection times. Although it is not possible to conclude a lack of sex effect on serum PA levels, given data for only 1 female, the lack of any apparent sex difference does justify inclusion of the female's results with those for the males that died in the subsequent analyses of serum PA kinetics.

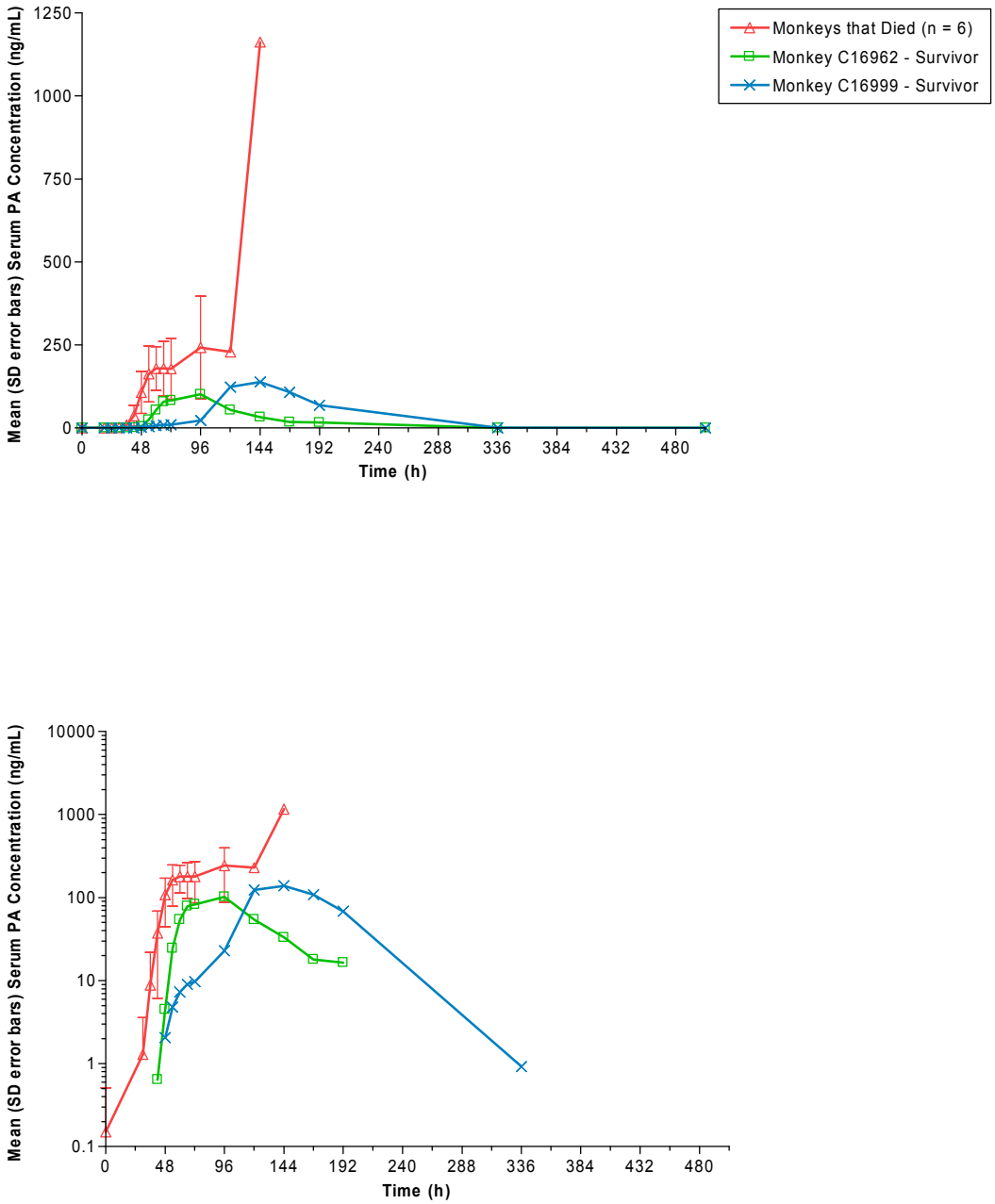

Figure 13-57 Serum PA concentration-time in monkeys (mean ± SD) (685-G005762)

(upper panel – linear plot; lower panel – semi-log plot).

The times to events in the serum PA concentration-time profile (t_{lag}, t_{1st}, t_{max}, $t_{plateau}$, and $t_{2nd\ rise}$) were compared with the times to relevant clinical observations (survival time, time to first detected bacteremia, time to confirmed bacteremia by PCR, and time to 1st consecutive increases in body temperature). Since t_{max} was the time of the last specimen collection before an animal died, t_{max} generally occurred within the 24 h preceding death. It should be noted that t_{1st} either coincides with the time to first detected bacteremia by culture in all but 1 of the animals that died, and for that animal serum PA was measurable at the next collection time. Bacteremia confirmed by PCR coincided with t_{1st} for all but 1 of the animals that died and for that animal serum PA became measurable at the collection time that preceded the time at which bacteremia was detected by PCR. The time to consecutive temperature increases could only be determined in 4 of the 6 monkeys that died, but for those monkeys, t_{1st} occurred 5 to 16 h earlier than the consecutive temperature elevations. Overall, these results indicate that the appearance of PA in serum is nearly coincident with appearance of bacteremia, and precedes the onset of increased temperature. Based on the results presented in Table 13-52, other serum PA kinetic parameters appear to be less closely associated with the clinical observations.

Table 13-52 Comparisons of serum PA concentration-time curve kinetic parameters with relevant clinical observations in monkeys that died (685-G005762)

	Time of Clinical Observation (h)	Elapsed Time Between Clinical Observation and Serum PA Concentration-Time Profile Events (h): t_{lag}	t_{1st}	t_{max}	$t_{plateau}$	$t_{2ndrise}$
N	6	6	6	6	6	4
			Survival Time			
Mean	115.8	87	81	17	65	48
Min	85.5	62	56	10	32	34
Median	110.3	83	77	16	65	49
Max	156.1	120	114	27	96	60
			Time to First Detected Bacteremia by Culture			
Mean	34	5	-1	-65	-17	-45
Min	30	0	-6	-102	-24	-60
Median	33	6	0	-63	-18	-48
Max	42	6	0	-36	-12	-24
			Time to Confirmed Bacteremia by PCR			
Mean	36	7	1	-63	-15	-44
Min	30	6	0	-102	-18	-54
Median	36	6	0	-63	-15	-48
Max	42	12	6	-36	-12	-24
			Time of First Consecutive Temperature Increases			
Mean	48	17	11	-66	-3	-33
Min	46	11	5	-97	-13	-49
Median	48	17	11	-60	-2	-36
Max	51	22	16	-48	4	-12

Abbreviations: t_{lag}, the time of the last serum PA concentration that was less than the lower limit of quantitation; t_{1st}, time to C_{1st}; t_{max}, time to C_{max}; $t_{plateau}$, time which marks the beginning of the plateau portion of the serum PA concentration-time profile; $t_{2nd\ rise}$, time at which the second increasing phase of the profile began.

13.16.2.2 Monkey Therapeutic Efficacy Study (Study 724-G005829) (AB50409.INF.0.040)

Total PA concentrations in serum samples were determined using an ECL-based bridging assay. The LLOQ is 0.65 ng/mL of PA in 100% monkey serum. Anti-PA antibody concentrations in serum samples were determined using an ELISA. The LLOQ of the assay is < 600 ng/mL of anti-PA antibody in 100% monkey serum. TNA titers in serum samples were determined using a cell killing assay. Blood specimens for serum PA kinetics were also to be collected from all monkeys at 3 days prior to spore challenge as well as at 24, 30, 36, 42, 48, and 54 hours after spore challenge, unless treatment occurred prior to the collection time. Additional blood specimens were collected just prior to raxibacumab or vehicle dosing; at

5 minutes after dosing; at 12 and 24 hours after dosing; and at 3, 5, 8, 14, and 28 days after dosing. When feasible, a terminal blood sample was to be taken just prior to euthanasia for animals that were judged to be moribund.

The individual serum PA concentration time profiles for animals that died and that survived within each treatment group are illustrated in Figure 13-58. Examination of the profiles for the individual animals that died in all treatment groups of the current study revealed serum PA concentration-time profiles generally followed the pattern of rise then plateau, with the exception that some animals died prior to attaining the plateau phase of the serum.

As shown in Figure 13-58, the serum PA concentration-time profiles for the animals that survived in the 20 and 40 mg/kg raxibacumab groups showed the initial rise, but before the plateau phase was attained, serum PA concentrations began to decline. Similarly, for the surviving animals in this study, the onset of declining serum PA concentrations was generally associated with attainment of negative bacteremia results. For all surviving animals in the 20 mg/kg dose group, serum PA concentrations declined to below the LLOQ by the end of the study. For the 40 mg/kg dose group, all but 1 of the 9 surviving animals attained serum PA concentrations below the LLOQ by the end of the study. In that dose group, 1 monkey had a low serum PA concentration of 0.720 ng/mL at the end of the study, which was nearly 7-fold lower than the concentration at the prior collection time.

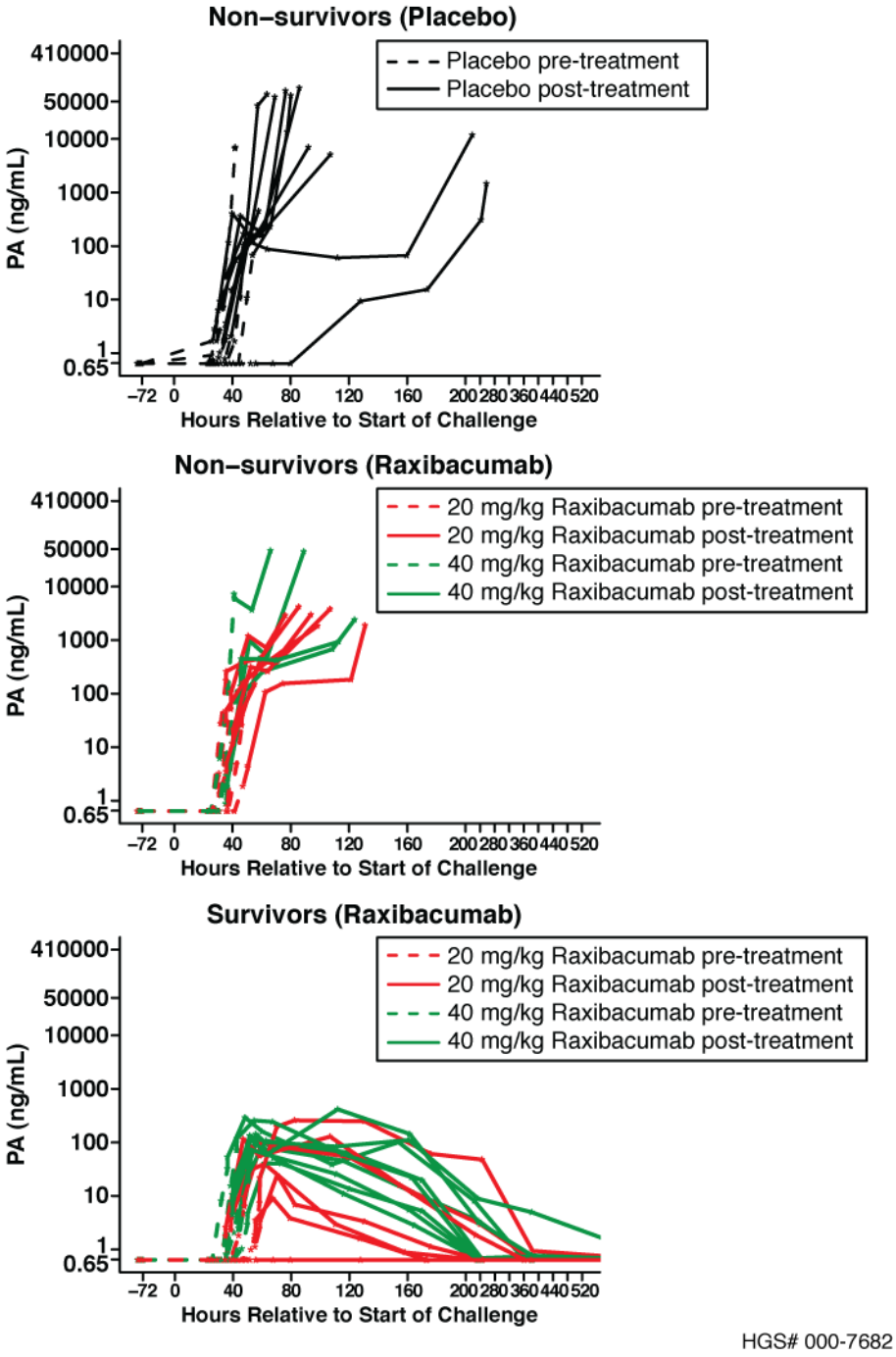

Figure 13-58 Serum PA profiles in monkeys (724-G005829)

Review of the serum PA concentration-time results for the surviving animals, in comparison with the results of bacteremia by culture tests, revealed that the decline in serum PA concentrations coincided with the sterilization of bacteremia after raxibacumab treatment. It cannot be distinguished if the sterilization of bacteremia is due to an unknown anti-bacterial effect of raxibacumab, or if it reflects the ability of the animals' immune response to sterilize bacteremia once the mortality-inducing effects of toxemia were neutralized by raxibacumab. Examination of the serum PA concentration-time profiles for the surviving monkeys in this study showed that for at least some monkeys, monotonically decreasing concentrations were present at post-raxibacumab treatment bacteremia-free collection times. Those profiles generally did not show increasing rates of clearance; hence, it seemed reasonable to assume that the clearance of PA from serum was not related to the development of anti-PA antibody in these animals. Therefore, PA $t_{1/2}$ was calculated for animals that had monotonically decreasing serum PA concentrations at times post raxibacumab dose, when the animal was bacteremia negative for each successive collection time. Individual PA $t_{1/2}$ results are summarized in Table 13-53. Estimation of $t_{1/2}$ was only possible for 4 surviving monkeys (1 in the 20 mg/kg dose group and 3 in the 40 mg/kg dose group). The overall mean $t_{1/2}$ for serum PA was 54 hours, with a median of 31 hours and range from 23 to 131 hours.

Table 13-53 PA elimination kinetics in surviving monkeys (724-G005829)

$t_{1/2}$ (h)	Mean	Median (Range)
Overall (n = 4)	54.18	31.17 (23.13 to 131.24)
For 20 mg/kg (n = 1)	29.59	NA
For 40 mg/kg (n = 3)	62.37	32.75 (23.13 to 131.24)

Abbreviations: $t_{1/2}$, PA elimination half-life; NA, not applicable.

The serum anti-PA antibody concentration results for the raxibacumab dose groups are summarized in Table 13-54. The mean serum anti-PA concentration for the 40 mg/kg dose group was more than 2-fold higher than that for the 20 mg/kg dose group. However, the inter-individual variation was large, such that the difference in the means did not attain statistical significance, as shown by the overlap in 95% CI, for the number of surviving animals in this study. Despite this, the results indicate that increasing exposure to raxibacumab did not decrease the formation of anti-PA antibody by the surviving monkeys in this study.

Table 13-54 Serum anti-PA antibody concentrations in surviving monkeys (724-G005829)

		Serum Anti-PA Antibody Concentration (µg/mL)	
		Predose	28 Day Postdose
20 mg/kg	N	7	7
	Mean	0	302
	95% CI	(0,0)	(-39,643)
40 mg/kg	N	9	9
	Mean	0	775
	95% CI	(0,0)	(292,1257)

The serum TNA titer results for the raxibacumab dose groups are summarized in Table 13-55. The mean serum TNA titer for the 40 mg/kg dose group was nearly 3-fold higher than that for the 20 mg/kg dose group. However, the inter-individual variation was large, such that the difference in the means did not attain statistical significance, as shown by the overlap in 95% CI, for the number of surviving animals in this study. As shown by the increase in mean serum TNA titer for the 40 mg/kg dose group relative to the 20 mg/kg group, increasing exposure to raxibacumab did not decrease the formation of TNA by the surviving monkeys in this study.

Table 13-55 Serum TNA titers in surviving monkeys (724-G005829)

		Serum TNA Titer	
		Predose	28 Day Postdose
20 mg/kg	N	7	7
	Mean	0	5328
	95% CI	(0,0)	(523,10133)
40 mg/kg	N	9	9
	Mean	0	14827
	95% CI	(0,0)	(5163,24490)

13.16.2.3 Monkey Raxibacumab/Ciprofloxacin Therapeutic Efficacy Study (Study 789-G923702) (AB50409.INF.0.042)

Total PA (free + raxibacumab-bound) concentrations in serum samples were determined using an ECL-based bridging assay. The LLOQ is 0.65 ng/mL of PA in 100% monkey serum. Anti-PA antibody concentrations in serum samples were determined using an ELISA. The LLOQ is < 600 ng/mL of anti-PA antibody in 100% monkey serum. TNA titers in serum samples were determined using a cell killing assay. The LLOQ is < 52 titer in 100% monkey serum.

Blood specimens for serum PA kinetics were also collected from all monkeys at 7 days prior to spore challenge as well as at 24, 30, 36, 42, 48, and 54 hours after spore challenge, unless treatment occurred prior to the collection time. Additional blood specimens were collected just prior to ciprofloxacin, raxibacumab, or vehicle dosing; at 5 minutes after dosing; at 24, 48, 72, and 120 hours after dosing; and at 8, 14, 21, and 28 days after spore challenge. When feasible, a terminal blood sample was taken just prior to euthanasia for animals that were judged to be moribund.

Blood specimens for serum anti-PA antibody and TNA were collected from all monkeys at 7 days prior to spore challenge as well as at 28 days after spore challenge. The blood specimens were allowed to clot and were then centrifuged. The serum was harvested, filtered, cultured for presence of *B. anthracis*, and stored at ≤ -70ºC prior to shipment to HGS) for assay. If sufficient serum volume for the 14 and 21 days post spore challenge specimens remained, those specimens may also have been analyzed for anti-PA antibody and TNA.

The mean (with SD error bars) observed serum PA concentration-time profiles for the placebo control group animals are illustrated in Figure 13-59. Examination of the profiles for the individual control group animals revealed serum PA concentration-time profiles generally followed the pattern of rise-plateau-rise, with the exception that some animals died prior to attaining either the plateau phase or the 2nd rising phase of the serum PA concentration-time profile. In a previous study to characterize the natural course of anthrax disease in monkeys (Study 685-G005762), serum PA concentration-time profiles from 8 monkeys challenged at ~200 x LD_{50} had a pattern of rise-plateau, with evidence of a 2nd rising phase in some animals, while in a prior study of raxibacumab efficacy (Study 724-G005829), monkeys symptomatic for inhalation anthrax generally had a pattern of rise-plateau.

As shown in Figure 13-60, the mean serum PA concentration-time profiles for the animals that survived in the ciprofloxacin and ciprofloxacin/raxibacumab groups showed the initial rise, but before or shortly after the plateau phase was attained, serum PA concentrations began to decline. This increasing then decreasing type of PA profile is generally characteristic of surviving animals, and is generally not observed in those animals that died. It should be noted that in a prior study to characterize the natural course of anthrax disease in monkeys (Study 685-G005762), similar serum PA concentration-time profiles (rising then declining) were observed in the 2 surviving animals. Rising then declining serum PA concentration-time profiles were also observed in the surviving animals from the raxibacumab efficacy study (Study 724-G005829). In those studies, the decline of serum PA levels was observed to generally coincide with the sterilization of bacteremia. Similarly, for the surviving animals in this study, the onset of declining serum PA concentrations was generally associated with attainment of negative bacteremia results. For all but 1 of the surviving animals, serum PA concentrations declined to below the LLOQ by 14 days post challenge and that animal had persistent low serum PA concentrations between 2 to 11 ng/mL throughout the duration of the study. As noted previously, this animal had a prechallenge serum PA level of 3.76 ng/mL, indicating that post-challenge serum PA concentrations had declined to levels similar to the prechallenge level by the end of the study. The low and persistent serum PA concentrations

account for the difference in mean serum PA concentration-time profiles between the 2 groups at the later timepoints.

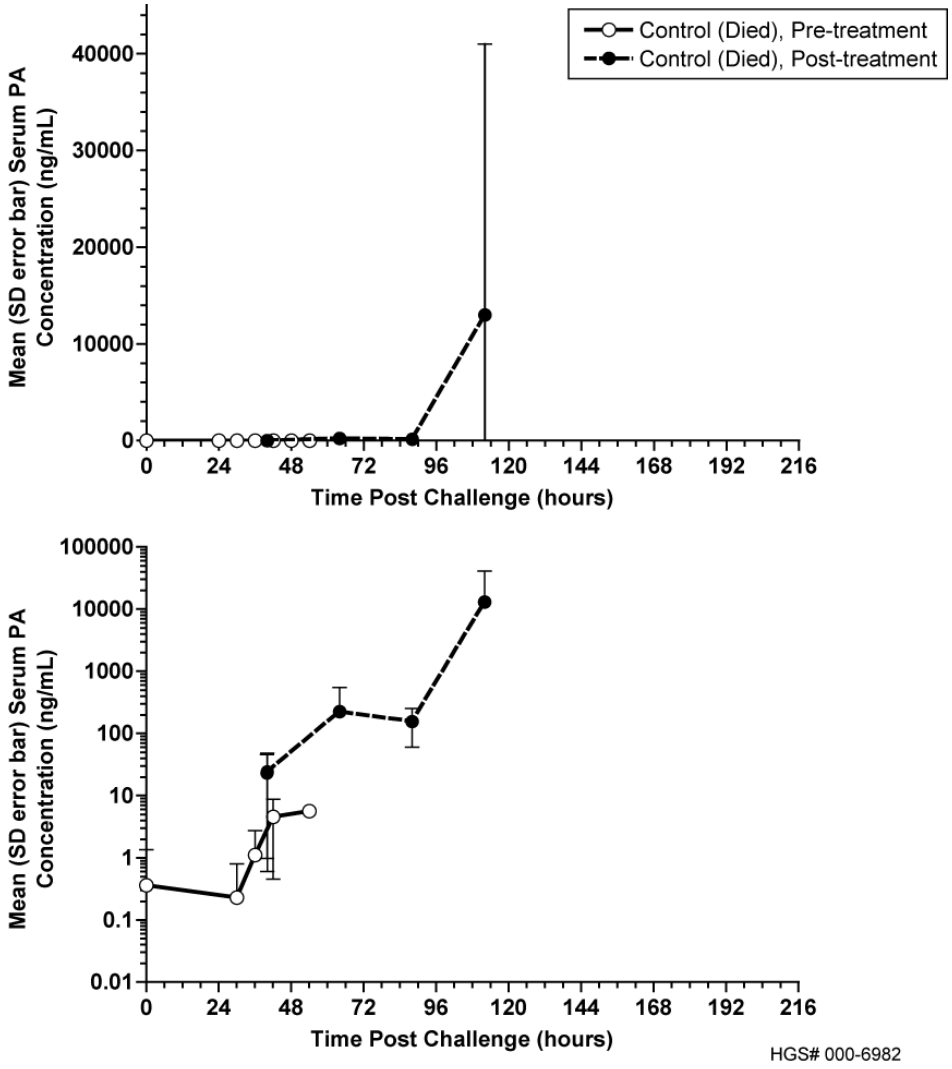

Figure 13-59 Serum PA concentration-time profiles in monkeys that died which were administered placebo

For plotting purposes, post-treatment collection times are expressed as times post challenge based on a typical treatment time of approximately 40 hours post challenge. Solid lines represent serum PA concentrations prior to treatment intervention, while dotted lines represent serum PA concentrations after treatment intervention.

Upper panel, linear scale; lower panel, semilog scale.

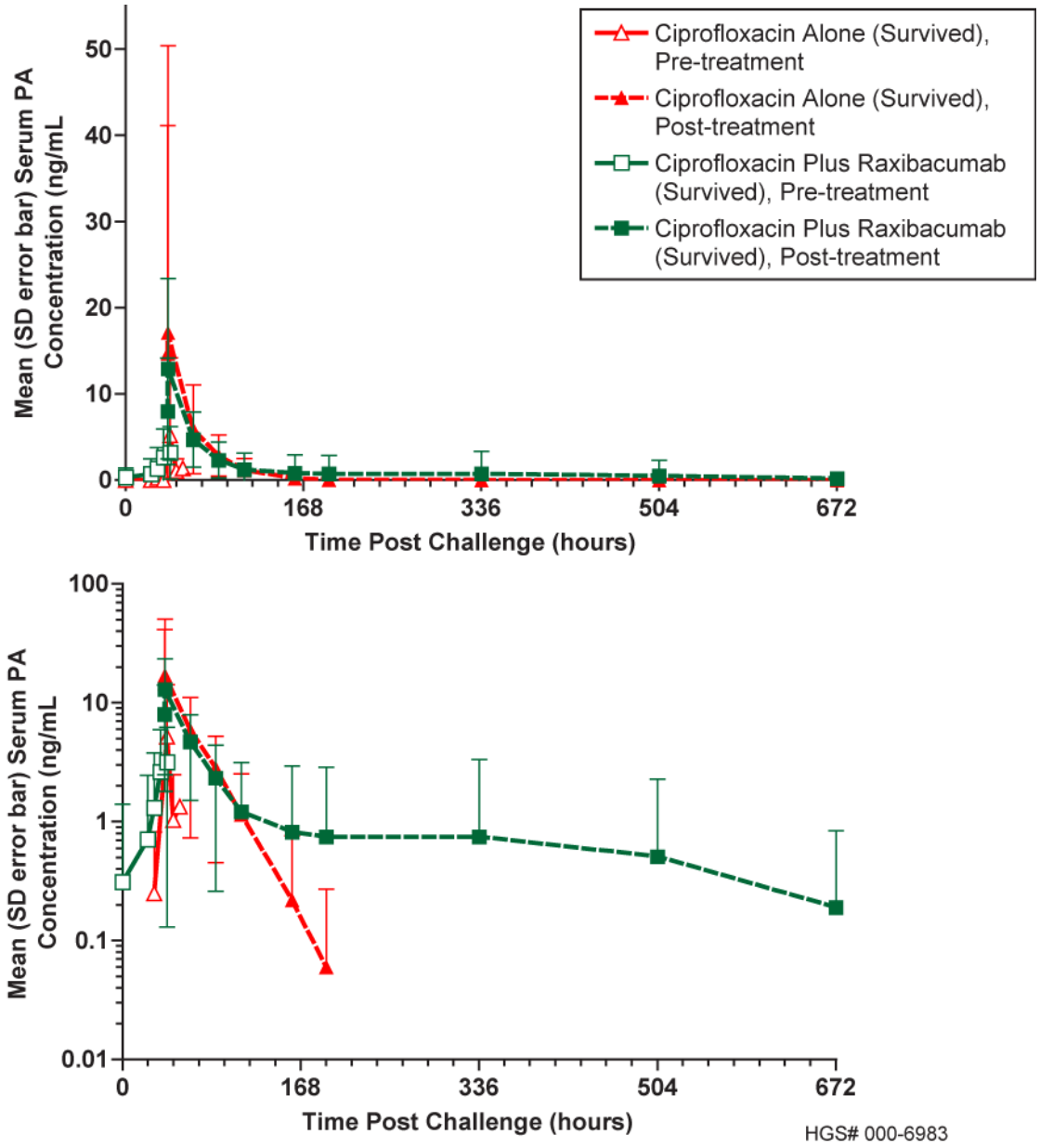

Figure 13-60 Serum PA concentration-time profiles in monkeys that survived (789-G923702)

For plotting purposes, post-treatment collection times are expressed as times post challenge based on a typical treatment time of approximately 40 hours post challenge. Solid lines represent serum PA concentrations prior to treatment intervention, while dotted lines represent serum PA concentrations after treatment intervention.

Upper panel, linear scale; lower panel, semilog scale.

As noted previously, for the surviving animals' serum PA concentrations declined after ciprofloxacin or ciprofloxacin/raxibacumab treatment. Review of the serum PA concentration-time results for the surviving animals, in comparison with the results of bacteremia by culture tests, revealed that the decline in serum PA concentrations coincided with the sterilization of bacteremia after treatment. Although sterilization of bacteremia is an expected effect of ciprofloxacin, it was also noted in a previous study when monkeys with inhalational anthrax were administered raxibacumab alone (Study 724-G005829). It is unknown if that reflects an anti-bacterial effect of raxibacumab, or if it reflects the ability of the animals' immune response to sterilize bacteremia once the mortality-inducing effects of toxemia were neutralized by raxibacumab. Examination of the serum PA concentration-time profiles for the surviving monkeys in this study showed that for at least some monkeys, monotonically decreasing concentrations were present at post-ciprofloxacin/raxibacumab treatment bacteremia-free collection times. Those profiles generally did not show increasing rates of PA clearance; hence, it seemed reasonable to assume that the clearance of PA from serum was not related to the development of anti-PA antibody in these animals. Anti-PA antibodies were observed by 21 days post spore challenge. Therefore, PA $t_{1/2}$ was calculated for animals that had monotonically decreasing serum PA concentrations at times post raxibacumab dose, when the animal was bacteremia negative for each successive collection time; furthermore, at the times used for calculation of PA $t_{1/2}$, the animals were anti-PA antibody negative. For the purposes of this analysis, if a bacteremia by culture result was not available for a collection time of interest, the bacteremia result of interest was imputed to be negative if the result at the preceding and subsequent collection times were negative; otherwise, the result was imputed to be positive.

The individual PA $t_{1/2}$ results are summarized in Table 13-56. Estimation of $t_{1/2}$ was only possible for 11 surviving monkeys in the ciprofloxacin alone dose group and 11 in the ciprofloxacin plus raxibacumab dose group. The mean $t_{1/2}$ for serum PA was 22 hours for the ciprofloxacin alone dose group, and was 71 hours for the ciprofloxacin plus raxibacumab dose group; however, the mean for the latter group is biased by 1 monkey and the median $t_{1/2}$ for serum PA were similar at 23 and 27 hours for the ciprofloxacin and ciprofloxacin/raxibacumab dose groups, respectively.

Table 13-56 PA elimination kinetics in surviving monkeys (789-G923702)

Parameter	Mean	Median (Range)
$t_{1/2}$ (h)		
For ciprofloxacin alone (n = 11)	22.09	22.72 (9.47 to 32.44)
For ciprofloxacin plus raxibacumab (n = 11)	70.71[1]	26.58 (16.09 to 431.42)

Abbreviations: $t_{1/2}$, PA elimination half-life; NA, not applicable.

[1] Value is skewed by the high result for 1 animal. The median represents the central tendency in this case.

The serum anti-PA antibody concentration results for the raxibacumab dose groups are summarized in Table 13-57. There were no statistically significant differences in mean serum

anti-PA antibody concentrations between the 2 treatment groups, indicating that co-administration of raxibacumab with ciprofloxacin did not affect the formation of anti-PA antibody by the surviving monkeys, relative to ciprofloxacin alone.

Table 13-57 Anti-PA serum antibody concentrations in surviving monkeys (789-G923702)

		Serum Anti-PA Antibody Concentration (µg/mL)		
		Predose	21 Day Postdose	28 Day Postdose
Ciprofloxacin	N	14	14	14
	Mean ± SD	0.068 ± 0.253	65.447 ± 37.687	89.126 ± 48.833
Ciprofloxacin/Raxibacumab	N	12	12	12
	Mean ± SD	0.295 ± 0.837	72.506 ± 33.779	100.899 ± 83.862
	P-value[1]	0.3819	0.6222	0.6600

[1] From an unpaired t-test.

The serum TNA titer results for the raxibacumab dose groups are summarized in Table 13-58. The mean serum TNA titers for the ciprofloxacin alone dose group were significantly higher (about 2-fold) than those for the ciprofloxacin plus raxibacumab dose group. It should be noted that these differences were not reflected in altered survival between the 2 groups, suggesting that the TNA response in monkeys administered both ciprofloxacin and raxibacumab was adequate for survival. The finding that anti-PA titers were high in the ciprofloxacinand ciprofloxacin/raxibacumab treatment groups (Table 13-57) confirms that both groups of animals could mount a rigorous innate immune response to PA.

Table 13-58 Serum TNA titers in surviving monkeys (789-G923702)

		Serum TNA Titer		
		Predose	21 Day Postdose	28 Day Postdose
Ciprofloxacin	N	14	14	14
	Mean ± SD	0 ± 0	6369 ± 5288	7266 ± 4864
Ciprofloxacin/Raxibacumab	N	12	12	12
	Mean ± SD	0 ± 0	3020 ± 1802	3400 ± 1869
	P-Value[1]	-	0.0405	0.0136

[1] From an unpaired t-test.

13.17 Appendix 17: Population PK for Raxibacumab and PA Kinetics

13.17.1 Raxibacumab PK in Humans

Population PK analyses were performed to develop a population PK model of serum raxibacumab concentrations in humans (healthy volunteers) and to identify and quantify the impact of covariates on raxibacumab PK in humans.

Three key assumptions were taken into consideration when evaluating the population PK data. First, it was assumed that the clinical studies provide data from a sufficiently diverse population to assure that the human raxibacumab PK can be generalized to the adult United States (US) population. Data from Study HGS1021-C1063, which constitutes the majority of the dataset for the population PK analysis, was conducted with the goal of attaining a study population representative of the US population. Second, it was assumed that efficacy of raxibacumab is primarily mediated by its binding of PA. While there is a possibility that raxibacumab may also exert an antibacterial effect, any such effect has not been quantified nor has an explicit mechanism been identified. Lastly, it was assumed that as an efficacy endpoint, mortality is associated with increasing circulating PA elaborated by the anthrax bacteria. A corollary to this assumption is that rapid attainment of sufficiently high serum raxibacumab concentrations is crucial for survival benefit, rather than prolonged exposure to relatively low serum raxibacumab levels; that is, C_{max} is more important for survival than AUC.

Raxibacumab serum concentrations for all subjects administered 40 mg/kg raxibacumab IV produced from the M11 BDS process and 21-A formulation proposed for licensure in the Phase 2/3 and Phase 3 studies in healthy subjects were included in the analysis as summarized in Table 13-59.

Table 13-59 Studies included in raxibacumab Population PK analysis in humans

Protocol No.	Phase	Study Description	Dose (mg/kg)	Number of Subjects
HGS1021-C1063	3	Safety and tolerability	40	238[1]
HGS1021-C1064	2/3	PK and safety when administered with ciprofloxacin	40	86
HGS1021-C1069	2/3	Immunogenicity and safety	40	20[2]
			Total	324

[1] Raxibacumab treated subjects only (ie, placebo-treated subjects are excluded).

[2] Excluded from total, since subjects also participated in HGS1021-C1064.

The population PK models assessed for serum raxibacumab concentrations included models with at least 2 compartments and 1st-order elimination. Error models for intersubject variability and residual error that was evaluated included additive, exponential, proportional, and combined additive/proportional error models. Potential covariates assessed for impact on raxibacumab PK included: age, sex, race, body weight, and laboratory assessments reflecting

renal or hepatic function. Prior to model building, a subset of data (~30% of the total data set, by random selection) was identified for use in evaluation, and was not utilized in the model building process. Model building was done in a stepwise fashion: 1st, the structural model was identified; next, error models of intersubject variability were developed; and then covariates were added to the model. In all cases, selection between competing models was based on goodness of fit criteria.

Model evaluation included prediction into the evaluation dataset; following successful demonstration of the predictive performance of the model, the full dataset was fit to the model to obtain the reported parameter values. The visual predictive check was used to assess how well the model described the full dataset. Precision of parameter estimates was characterized by log likelihood profiling. Stability of the parameter estimates and sensitivity to subgroups of subjects were assessed using leverage analysis.

Human serum raxibacumab concentrations for 2229 post-dose specimens from 322 subjects (47% male, 53% female; 71% Caucasian, 16% Black, 5% Asian; 84% non-Hispanic, 16% Hispanic; age range 18 to 87 years [median 37 years]; body weight range 45 to 156 kg [median 76 kg]) best fit a 2-compartment open model with 1st-order elimination from the central compartment.

The final model was used to predict into the test data, which were not used for model building. There was good agreement between the observations in the test data set and predicted values from the final model. The observed and predicted serum raxibacumab concentrations for the subjects indicated that the final model provides unbiased and satisfactorily precise predictions, and the full dataset was fit to the model to obtain the PK parameter estimates for the whole population.

Population values were 180 mL/day for CL, 3312 mL for V_1, 487 mL/day for CLD_2, and 2243 mL for V_2 (Table 13-60). Body weight was a significant covariate for all PK parameters, with parameter values increasing 2- to 4-fold across the weight range for the population. Although sex and Black race were covariates for V_1 and CLD_2, respectively, the impact of these covariates was much smaller than that for weight, at 11% and 3%, respectively, suggesting these covariates are not clinically meaningful. The model predicted median serum raxibacumab concentrations at peak and at 28 days post-dose of 903 and 181 µg/mL, respectively.

Table 13-60 Raxibacumab PK parameters (Population PK)

Primary Parameters	Value (RSE [%])	CV% (RSE [%])
V_1 (mL)	3312 (1.4)	16.0 (9.8)
Effect of sex on V_1 (mL)[1]	V_1 = 3312 x (1 + (-0.0992 x sex))	(20.1)
Males	3312	
Females	2984	
Effect of weight on V_1 (mL)[2]	V_1 = 3312 + (26.3 x (weight – 74))	(8.3)
At 45 kg	2549	
At 62 kg	2996	
At 86 kg	3628	

Table 13-60 Raxibacumab PK parameters (Population PK)

Primary Parameters	Value (RSE [%])	CV% (RSE [%])
At 156 kg	5469	
CL (mL/day)	180 (1.2)	20.0 (10.6)
Effect of weight on CL (mL/day)[2]	CL = 180 + (2.02 x (weight – 74))	(6.7)
At 45 kg	122	
At 62 kg	156	
At 86 kg	205	
At 156 kg	346	
V_2 (mL)	2243 (1.9)	21.2 (33.3)
Effect of weight on V_2 (mL)[2]	V_2 = 2243 + (26 x (weight – 74))	(7.8)
At 45 kg	1489	
At 62 kg	1931	
At 86 kg	2555	
At 156 kg	4374	
CLD_2 (mL/day)	487 (5.4)	33.4 (35.4)
Effect of race = Black on CLD_2 (mL/day)	CLD_2 = 487 x (1 + -0.0328) if race = Black	(426.4)
Black	471	
Non-Black	487	
Effect of weight on CLD_2 (mL/day)[2]	CLD_2 = 487 + (7.98 x (weight – 74))	(8.2)
At 45 kg	256	
At 62 kg	391	
At 86 kg	583	
At 156 kg	1142	
Residual Variability	9.5% CV% for proportional error component (10.0) 9.4 µg/mL SD for additive error component (25.8)	

Abbreviations: CV%, coefficient of variation; V_1, volume of distribution for the central compartment; CL, clearance; V_2, volume of distribution for the peripheral compartment; CLD_2, intercompartmental clearance; RSE, relative standard error.

[1] For sex coded as 0 = male and 1 = female.

[2] The median weight for model building data set was used for centering.

(concluded)

Simulations were performed to assess the impact of sex (covariate for V_1) and Black race (covariate for CLD_2) on secondary (derived) PK parameters. As shown in Table 13-61. The maximum serum raxibacumab concentrations were 11% higher in females than in males. Since CL was unaffected by sex or race, the $AUC_{0-\infty}$ did not differ among the subgroups. There were small differences in the $t_{1/2,\alpha}$ due to sex (< 6%) or due to race (< 3%). Similarly, there were minimal differences in the $t_{1/2,\beta}$ due to sex (< 6%) or due to race (< 1%). There were minimal differences in mean residence times (MRT) or V_{ss} due to sex (< 7%), with no differences due to race. Overall, this assessment indicates that neither sex nor race have a clinically meaningful impact on raxibacumab PK.

Table 13-61 Secondary raxibacumab PK parameters by sex and race (Population PK)

	Non-Black		Black	
Secondary Parameters[1]	**Males**	**Females**	**Males**	**Females**
C_{max} (µg/mL)	886	983	886	983
$AUC_{0-\infty}$ (µg·day/mL)	16393	16393	16393	16393
$t_{1/2,\alpha}$ (days)	1.79	1.70	1.84	1.75
$t_{1/2,\beta}$ (days)	22.73	21.56	22.78	21.61
MRT (days)	30.77	28.95	30.77	28.95
V_{ss} (mL/kg)	75.07	70.63	75.07	70.63

Abbreviations: C_{max}, maximum serum drug concentration; $AUC_{0-\infty}$, area under the serum drug concentration-time curve from time 0 to infinite time; $t_{1/2,\alpha}$, elimination half-life for the 1st phase; $t_{1/2,\beta}$, elimination half-life for the 2nd (terminal) phase; MRT, mean residence time; V_{ss}, volume of distribution at steady-state.

[1] Assuming the weight of 74 kg (median weight for model building data set).

Simulations also were performed to assess the impact of weight on secondary PK parameters. As shown in Table 13-62, C_{max} increased by 61% from 45 to 156 kg, while $AUC_{0-\infty}$ increased by 22% over the weight range. The values of $t_{1/2,\alpha}$ decreased by 42%, whereas $t_{1/2,\beta}$ and MRT both decreased by 15% as weight increased from 45 to 156 kg., V_{ss} also decreased by 30% as weight increased. Overall, these results show that weight has substantial impact on raxibacumab PK.

Table 13-62 Secondary raxibacumab PK parameters by body weight (Population PK)

Secondary Parameters	**45 kg**	**62 kg**	**74 kg**	**86 kg**	**156 kg**
C_{max} (µg/mL)	702	821	886	940	1128
$AUC_{0-\infty}$ (µg·day/mL)	14760	15873	16393	16807	18018
$t_{1/2,\alpha}$ (days)	2.38	1.95	1.79	1.67	1.39
$t_{1/2,\beta}$ (days)	24.61	23.33	22.73	22.30	20.97
MRT (days)	33.11	31.54	30.77	30.21	28.42
V_{ss} (mL/kg)	89.74	79.47	75.07	71.90	63.10

Abbreviations: C_{max}, maximum serum drug concentration; $AUC_{0-\infty}$, area under the serum drug concentration-time curve from time 0 to infinite time; $t_{1/2,\alpha}$, elimination half-life for the 1st phase; $t_{1/2,\beta}$, elimination half-life for the 2nd (terminal) phase; MRT, mean residence time; V_{ss}, volume of distribution at steady-state.

The post hoc PK parameter estimates are summarized in Table 13-63. For comparison, the table also includes the noncompartmental PK analysis results for 40 mg/kg IV raxibacumab doses from the human clinical studies, PAM-NH-01, HGS1021-C1064, and HGS1021-C1069. There was good agreement in PK among the studies, indicating that the population PK analysis results are consistent with the results for the prior studies.

Table 13-63 Raxibacumab PK parameters by study (Population PK)

	Population Analysis[1] (n = 322)	PAM-NH-01 (n = 7)	HGS1021-C1064[2] (n = 28)	HGS1021-C1069 (n = 20)
C_{max} (µg/mL)	960 ± 164	1042 ± 88	988 ± 220	979 ± 148
$AUC_{0-\infty}$ (µg·day/mL)	16667 ± 3198	15554 ± 3273	15328 ± 5059	18239 ± 6179
$t_{1/2,\alpha}$ (days)	1.76 ± 0.36	NA	NA	NA
$t_{1/2,\beta}$ (days)	22.35 ± 4.04	16.21 ± 2.30	20.44 ± 6.46	25.68 ± 11.19
MRT (days)	30.09 ± 5.76	22.20 ± 3.65	27.30 ± 8.24	35.09 ± 15.58
CL (mL/day/kg)	2.49 ± 0.49	2.64 ± 0.48	2.85 ± 1.03	2.37 ± 0.63
CLD_2 (mL/day/kg)	6.56 ± 0.91	NA	NA	NA
V_1 (mL/kg)	42.86 ± 7.28	NA	NA	NA
V_2 (mL/kg)	30.06 ± 4.34	NA	NA	NA
V_{ss} (mL/kg)	72.92 ± 10.07	57.6 ± 5.2	71.74 ± 17.36	75.72 ± 11.42

Abbreviations: C_{max}, maximum serum drug concentration; $AUC_{0-\infty}$, area under the serum drug concentration-time curve from time 0 to infinite time; $t_{1/2,\alpha}$, elimination half-life for the 1st phase; $t_{1/2,\beta}$, elimination half-life for the 2nd (terminal) phase; MRT, mean residence time; CL, clearance; CLD_2, intercompartmental clearance; V_1, volume of distribution for the central compartment; V_2, volume of distribution for the peripheral compartment; V_{ss}, volume of distribution at steady-state; NA, not available.

1 Mean and SD of individual subjects' post hoc estimates are presented.

2 For group treated with raxibacumab alone.

The population PK model was evaluated using a visual predictive check and separate predictive checks were performed for subjects that were administered a single raxibacumab dose, subjects that were administered 2 raxibacumab doses 14 days apart, and subjects that were administered 2 raxibacumab doses at least 4 months apart. The majority of the observed serum raxibacumab concentrations were within the 90% prediction interval, suggesting that the model describes the data well, and can be used to satisfactorily predict the range of concentrations for this population of subjects.

The precision of the population PK model parameter estimates was assessed by log likelihood profiling. The 95% CI bounds for the model parameters are summarized in Table 13-64. The 95% CI were relatively narrow, showing that the parameters were satisfactorily precise. It is worth noting that the 95% CI for the effect of Black race on CLD_2 includes 0, implying that effect is not significant, consistent with the prior results suggesting this is not a clinically important effect.

Table 13-64 Raxibacumab PK parameters with 95% CI by log likelihood profiling

Parameters	Value	95% CI
V_1 (mL)	3312	3215, 3412
Effect of sex on V_1 (mL)	-0.0992	-0.1356, -0.0607
Effect of weight on V_1 (mL)	26.3	22.1, 30.5
CL (mL/day)	180	176, 185
Effect of weight on CL (mL/day)	2.02	1.75, 2.29
V_2 (mL)	2243	2153, 2335
Effect of weight on V_2 (mL)	26	20, 32
CLD_2 (mL/day)	487	428, 562
Effect of race = Black on CLD_2 (mL/day)	-0.0328	-0.2079, 0.2003
Effect of weight on CLD_2 (mL/day)	7.98	4.05, 12.47

Abbreviations: V_1, volume of distribution for the central compartment; CL, clearance; V_2, volume of distribution for the peripheral compartment; CLD_2, intercompartmental clearance; CI, confidence interval.

13.17.2 Population PA Kinetics in Rabbits and Monkeys

Modeling of the kinetics of PA was performed with data from anthrax-spore challenged NZW rabbits and cynomolgus monkeys that received either no treatment (ie, model characterization studies 615-N104504 and 685-G005762 and the levofloxacin pilot PK study 723-G005835) or placebo treatment (ie, efficacy studies 682-G005758, 724-G005829, 781-G923701, and 789-G923702). PA kinetics was analyzed separately for rabbits and monkeys (Table 13-65).

Table 13-65 Summary of studies included in population PA kinetics in rabbits and monkeys

Study	Species	Study Description	Target Spore Challenge (x LD_{50})		Number of Animals[1]
615-N104504	Rabbit	Characterization of disease natural history	200		8[2]
682-G005758	Rabbit	Efficacy of raxibacumab	200		18
723-G005835	Rabbit	Pilot evaluation of levofloxacin efficacy and PK	200		3
781-G923701	Rabbit	Levofloxacin/raxibacumab interaction	200		12
				Total	38
685-G005762	Monkey	Characterization of disease natural history	200		8
724-G005829	Monkey	Efficacy of raxibacumab	200		12
789-G923702	Monkey	Ciprofloxacin/raxibacumab interaction	200		12
				Total	30

[1] For control group only (ie, no active treatment was administered).

[2] One rabbit in this study did not die during the index study period and was excluded from the PA kinetic analysis.

Kinetic analyses of total PA concentration-time profiles were conducted using population analysis techniques, with the NONMEM software. The serum PA concentration-time profiles comprise 3 phases: an initial rapid rise, followed by a plateau period of more slowly increasing or even decreasing levels, and a terminal phase during which levels increased rapidly again. The first 2 phases of the profile are consistent with the Gompertz equation that is used to describe biological growth, which displays a lag phase, followed by an exponential growth phase, which then approaches an asymptote. Many bacteria display a diauzic growth pattern that consists of an initial phase as described by the Gompertz model, but following establishment of the asymptotic plateau, a new exponential growth phase begins, followed by a terminal asymptotic phase. Liquori, et al (1981) modeled diauxic growth as the sum of 2 separate weighted growth functions. This function was applied to the diauxic serum PA concentration-time profiles, using the modified Gompertz equation of Zwietering, et al (1990) to describe the 2 phases of the growth curve. For the PA kinetics modeling, data for animals that died could be fit to the diauxic Gompertz model. Potential covariates assessed for impact on PA kinetics included: age, sex, body weight, size of *B. anthracis* spore challenge, duration of *B. anthracis* spore challenge, and time to 1st positive bacteremia (by culture).

Model building was done in a stepwise fashion: 1st, the structural model was identified; next, error models of intersubject variability were developed; and then covariates were added to the model. In all cases, selection between competing models was based on goodness of fit criteria.

A visual predictive check was used to assess how well the model described the data. Precision of parameter estimates was characterized by log likelihood profiling. Stability of the parameter estimates and sensitivity to individual animal's results were assessed using leverage analysis.

The population PA kinetics analysis was performed using data for 344 specimens from 38 rabbits that were obtained in 4 studies (615-N104504, 682-G005758, 723-G005835, and 781-G923701). All animals used for the PA kinetics analysis were not treated with either raxibacumab or antibiotics, had been exposed to a target 200 x LD_{50} *B. anthracis* inhalation spore challenge and died or were euthanized during the index study period. Of the 38 rabbits, 20 (53%) were male and 18 (47%) were female. The rabbits had body weights within a relatively narrow range (2.70 to 3.85 kg). On average, detection of bacteremia by culture or toxemia by detectable PA occurred 52 to 44 h prior to death in these rabbits that did not receive either raxibacumab or antibiotic.

The population model parameters included N_0, PA concentration at time 0; A, natural log of the ratio of the PA concentration in the asymptotic phase to N_0; μ_m, maximum specific growth rate for the 1st phase; λ, lag time for the 1st phase; λ_2, lag time for the 2nd growth phase; and $\mu_{m,2}$, maximum specific growth rate for the 2nd phase. Covariates included time to 1st positive bacteremia by culture (TBAC); body weight (WT); and survival time (TSUR). The parameters estimates are summarized in Table 13-66. Population values were 1.43 ng/mL for N_0, 22.3 h for λ, 0.775 h^{-1} for μ_m, 4.34 for A, 43.4 h for λ_2, and 0.335 h^{-1} for $\mu_{m,2}$. Body weight was a significant covariate for μ_m, with parameter values decreased nearly 3-fold across the weight range for the population. Survival time was related to A, λ_2, and $\mu_{m,2}$. As survival time

increased across a nearly 4-fold range, A decreased by about 6-fold, there was a 70% increase in λ_2, and $\mu_{m,2}$ decreased by 7-fold. In other words, increased survival time was associated with lower plateau concentrations, a longer duration of the plateau phase, and a slower rate of rise in the 2nd rising phase. Time to 1st positive bacteremia by culture was associated with λ; as λ increased, time to 1st bacteremia increased. The relative standard error (RSE) for the estimates of the covariate effects were less than 30%.

Table 13-66 PA kinetic parameters in rabbits (population PK)

Primary Parameters	Value (RSE [%])	CV% (RSE [%])
N_0 (ng/mL)	1.43 (68.72)	114.4 (39.0)
λ (h)	22.3 (4.15)	6.3 (59.6)
Effect of time to 1st positive bacteremia by culture (TBAC) on λ (h)	$\lambda(TBAC/24)^{1.09}$	(6.93)
At 19.39 h	17.7	
At 24 h	22.3	
At 94.85 h	99.6	
μ_m (h^{-1})	0.775 (3.41)	-
Effect of weight (WT) on μ_m (h^{-1})	$\mu_m - 0.518\ (WT - 3.2)$	(20.67)
At 2.70 kg	1.034	
At 3.24 kg	0.754	
At 3.85 kg	0.438	
A (unitless)	4.34 (18.86)	30.5 (6.9)
Effect of survival time (TSUR) on A	$A(TSUR/72.83)^{-1.47}$	(20.39)
At 33.27 h	13.71	
At 71.25 h	4.49	
At 116.50 h	2.18	
λ_2 (h)	43.4 (3.89)	6.0 (59.58)
Effect of survival time (TSUR) on λ_2 (h)	$\lambda_2(TSUR/72.83)^{0.426}$	(30.08)
At 33.27 h	31.13	
At 71.25 h	43.04	
At 116.50 h	53.06	
$\mu_{m,2}$ (h^{-1})	0.335 (7.74)	-
Effect of survival time (TSUR) on $\mu_{m,2}$ (h^{-1})	$\mu_{m,2}(TSUR / 72.83)^{-1.58}$	(15.10)
At 33.27 h	1.150	
At 71.25 h	0.346	
At 116.50 h	0.160	
Residual variability (CV%)	44.7 (18.6)	

Abbreviations: CV%, coefficient of variation; N_0, PA concentration at time 0; A, natural log of the ratio of the PA concentration in the asymptotic phase to N_0; μ_m, maximum specific growth rate for the 1st phase; λ, lag time for the 1st phase; λ_2, lag time for the 2nd growth phase; $\mu_{m,2}$, maximum specific growth rate for the 2nd phase; TBAC, time to 1st positive bacteremia by culture; WT, body weight; TSUR, survival time; RSE, relative standard error.

The post hoc PA kinetic parameter estimates for individual rabbits are summarized in Table 13-67.

Table 13-67 PA kinetic parameters in rabbits (mean ± SD) (Population PK)

	Population Analysis[1] (n = 39)
N_0 (ng/mL)	3.596 ± 10.493
λ (h)	30.6 ± 19.2
μ_m (h^{-1})	0.753 ± 0.153
A (unitless)	4.77 ± 2.64
λ_2 (h)	44.0 ± 5.4
$\mu_{m,2}$ (h^{-1})	0.359 ± 0.179

Abbreviations: N_0, PA concentration at time 0; A, natural log of the ratio of the PA concentration in the asymptotic phase to N_0; μ_m, maximum specific growth rate for the 1st phase; λ, lag time for the 1st phase; λ_2, lag time for the 2nd growth phase; $\mu_{m,2}$, maximum specific growth rate for the 2nd phase.

[1] Mean and SD of individual subjects' post hoc estimates are presented.

The population PA kinetics model was evaluated using a visual predictive check. Separate predictive checks were performed for rabbits with survival times less than 55 hours, for rabbits with survival times greater than or equal to 55 hours but less than 79 hours, and for rabbits with survival times greater than or equal to 79 hours. The vast majority of the observed serum raxibacumab concentrations was within the 90% prediction interval, suggesting that the model describes the data well, and can be used to satisfactorily predict the range of concentrations for this population of subjects. Despite this, the uncertainty of the predictions is substantial, as can be noted from the wide 90% prediction intervals.

The precision of the population PA kinetic model parameter estimates was assessed by log likelihood profiling. The 95% CI bounds for the model parameters are summarized in Table 13-68. The 95% CI indicate that the parameters were adequately precise.

Table 13-68 Serum or plasma PA kinetics in rabbits (Population PK)

Parameters	Value	95% CI
N_0 (ng/mL)	1.43	0.95, 2.25
λ (h)	22.3	21.48, 23.06
μ_m (h^{-1})	0.775	0.728, 0.826
A (unitless)	4.34	3.64, 4.91
λ_2 (h)	43.4	43.14, 43.69
$\mu_{m,2}$ (h^{-1})	0.335	0.333, 0.338
Effect of time to 1st positive bacteremia by culture (TBAC) on λ (h)	1.09	0.985, 1.184
Effect of weight (WT) on μ_m (h^{-1})	-0.518	-0.702, -0.309
Effect of survival time (TSUR) on A	-1.47	-1.941, -0.987
Effect of survival time (TSUR) on λ_2 (h)	0.426	0.330, 0.518
Effect of survival time (TSUR) on $\mu_{m,2}$ (h^{-1})	-1.58	-1.606, -1.548

Abbreviations: CV%, coefficient of variation; N_0, PA concentration at time 0; A, natural log of the ratio of the PA concentration in the asymptotic phase to N_0; μ_m, maximum specific growth rate for the 1st phase; λ, lag time for the 1st phase; λ_2, lag time for the 2nd growth phase; $\mu_{m,2}$, maximum specific growth rate for the 2nd phase; TBAC, time to 1st positive bacteremia by culture; TSUR, survival time.

The population PA kinetics analysis was performed using data for 265 specimens from 30 monkeys that were obtained in 3 studies (685-G005762, 724-G005829, and 789-G923702). All animals used for the PA kinetics analysis were not treated with either raxibacumab or antibiotics, had been exposed to a target 200 x LD_{50} *B. anthracis* inhalation spore challenge, and died or were euthanized during the index study period. Of the 30 monkeys, 13 (43%) were male and 17 (57%) were female. The monkeys had ages ranging from 3.0 to 6.8 years, and body weights ranging from 2.6 to 7.7 kg. On average, detection of bacteremia either by culture or toxemia by detectable PA occurred about 64 h prior to death in these monkeys that did not receive either raxibacumab or antibiotic.

The population model parameter estimates are summarized in Table 13-69. Population values were 1.21 ng/mL for N_0, 35.0 h for λ, 0.515 h^{-1} for μ_m, 4.94 for A, 71.3 h for λ_2, and 0.474 h^{-1} for $\mu_{m,2}$. Survival time was related to λ_2 and $\mu_{m,2}$: as survival time increased across a nearly 6-fold range, there was nearly a 6-fold increase in λ_2, and $\mu_{m,2}$ decreased by 7-fold. In other words, increased survival time was associated with a longer duration of the plateau phase and a slower rate of rise in the 2nd rising phase. Time to 1st positive bacteremia by culture was associated with λ; as λ increased, time to 1st bacteremia increased.

Table 13-69 PA kinetic parameters in monkeys (Population PK)

Parameters	Value (RSE [%])	CV% (RSE [%])
N_0 (ng/mL)	1.21 (16.5)	23.5 (126.8)
λ (h)	35.0 (1.8)	7.2 (36.9)
Effect of time to 1st positive bacteremia by culture (TBAC) on λ (h)	λ + 0.8779(TBAC – 35.72)	(6.4)
At 26.00 h	26.5	
At 35.72 h	35.0	
At 128.14 h	116.2	
μ_m (h^{-1})	0.515 (9.0)	41.4 (59.0)
A (unitless)	4.94 (3.7)	20.3 (74.3)
λ_2 (h)	71.3 (2.1)	0.001 (4142.6)
Effect of survival time (TSUR) on λ_2 (h)	λ_2 + 0.7979(TSUR – 93)	(3.6)
At 46.20 h	34.0	
At 92.79 h	71.2	
At 257.89 h	202.9	
$\mu_{m,2}$ (h^{-1})	0.474 (8.1)	11.5 (55.2)
Effect of survival time (TSUR) on $\mu_{m,2}$ (h^{-1})	$\mu_{m,2}(TSUR / 93)^{-1.11478}$	(10.1)
At 46.20 h	1.034	
At 92.79 h	0.475	
At 257.89 h	0.152	
Residual variability (CV%)	28.6 (21.9)	

Abbreviations: CV%, coefficient of variation; N_0, PA concentration at time 0; A, natural log of the ratio of the PA concentration in the asymptotic phase to N_0; μ_m, maximum specific growth rate for the 1st phase; λ, lag time for the 1st phase; λ_2, lag time for the 2nd growth phase; $\mu_{m,2}$, maximum specific growth rate for the 2nd phase; TBAC, time to 1st positive bacteremia by culture; TSUR, survival time; RSE, relative standard error.

The post hoc PA kinetic parameter estimates for individual monkeys are summarized in Table 13-70.

Table 13-70 PA kinetic parameters in monkeys (mean ± SD) (Population PK)

	Population Analysis[1] (n = 30)
N_0 (ng/mL)	1.19 ± 0.14
λ (h)	39.3 ± 17.3
μ_m (h^{-1})	0.558 ± 0.174
A (unitless)	5.07 ± 1.14
λ_2 (h)	81.4 ± 36.9
$\mu_{m,2}$ (h^{-1})	0.448 ± 0.196

Abbreviations: N_0, PA concentration at time 0; A, natural log of the ratio of the PA concentration in the asymptotic phase to N_0; μ_m, maximum specific growth rate for the 1st phase; λ, lag time for the 1st phase; λ_2, lag time for the 2nd growth phase; $\mu_{m,2}$, maximum specific growth rate for the 2nd phase.

[1] Mean and SD of individual subjects' post hoc estimates are presented.

The population PA kinetics model was evaluated using a visual predictive check. Separate predictive checks were performed for monkeys with survival times less than 90 hours, monkeys with survival times greater than or equal to 90 h but less than 150 hours, and for monkeys with survival times greater than or equal to 150 hours. The vast majority of the observed serum raxibacumab concentrations are within the 90% prediction interval, suggesting that the model describes the data well, and can be used to satisfactorily predict the range of concentrations for this population of subjects. Despite this, the uncertainty of the predictions is substantial, as can be noted from the wide 90% prediction intervals.

The precision of the population PA kinetic model parameter estimates was assessed by log likelihood profiling. The 95% CI bounds for the model parameters are summarized in Table 13-71 and indicate that the parameters were adequately precise.

Table 13-71 Serum/plasma PA kinetics in monkeys (Poulation PK)

Parameters	Value	95% CI
N_0 (ng/mL)	1.21	1.00, 1.44
λ (h)	35.05	34.09, 36.05
μ_m (h^{-1})	0.515	0.434, 0.568
A (unitless)	4.94	4.57, 5.35
λ_2 (h)	71.34	70.91, 71.78
$\mu_{m,2}$ (h^{-1})	0.474	0.462, 0.483
Effect of time to 1st positive bacteremia by culture (TBAC) on λ (h)	0.8779	0.6328, 1.0000
Effect of survival time (TSUR) on λ_2 (h)	0.7979	0.7800, 0.8072
Effect of survival time (TSUR) on $\mu_{m,2}$ (h^{-1})	1.11478	0.99662, 1.22747

Abbreviations: CV%, coefficient of variation; N_0, PA concentration at time 0; A, natural log of the ratio of the PA concentration in the asymptotic phase to N_0; μ_m, maximum specific growth rate for the 1st phase; λ, lag time for the 1st phase; λ_2, lag time for the 2nd growth phase; $\mu_{m,2}$, maximum specific growth rate for the 2nd phase; TBAC, time to 1st positive bacteremia by culture; TSUR, survival time.

13.17.3 Assessment of Protective Raxibacumab Exposure in Humans

The section examines PA concentration-time profiles and raxibacumab PK parameters in animals that survived and did not survive anthrax disease as an approach to assess the amount of raxibacumab that provides effective protection against the toxic effects of PA.

The serum/plasma PA concentration-time profiles for *B. anthracis* inhalation spore-challenged animals that were untreated and died (model characterization studies) or that were administered placebo and died (efficacy studies) are contrasted with the profile for animals that survived, in Figure 13-61 for rabbits and in Figure 13-62 for monkeys. Both figures illustrate the fundamental difference in the time course of serum/plasma PA concentrations in animals that survived vs untreated animals that died: in surviving animals, serum/plasma PA concentrations reached a peak, but then decreased, whereas in animals that died, serum/plasma PA levels continued to increase until death. In addition, the peak serum/plasma PA levels in animals that died were orders of magnitude higher than the peak levels in the animals that survived. The difference in serum/plasma PA profiles between animals that died and those that survived is relevant for assessing of the efficacy of a human raxibacumab dose. These data show that therapeutic interventions can block the progression of serum/plasma PA concentrations to the high levels associated with death, and that once this is accomplished, serum/plasma PA levels will decrease.

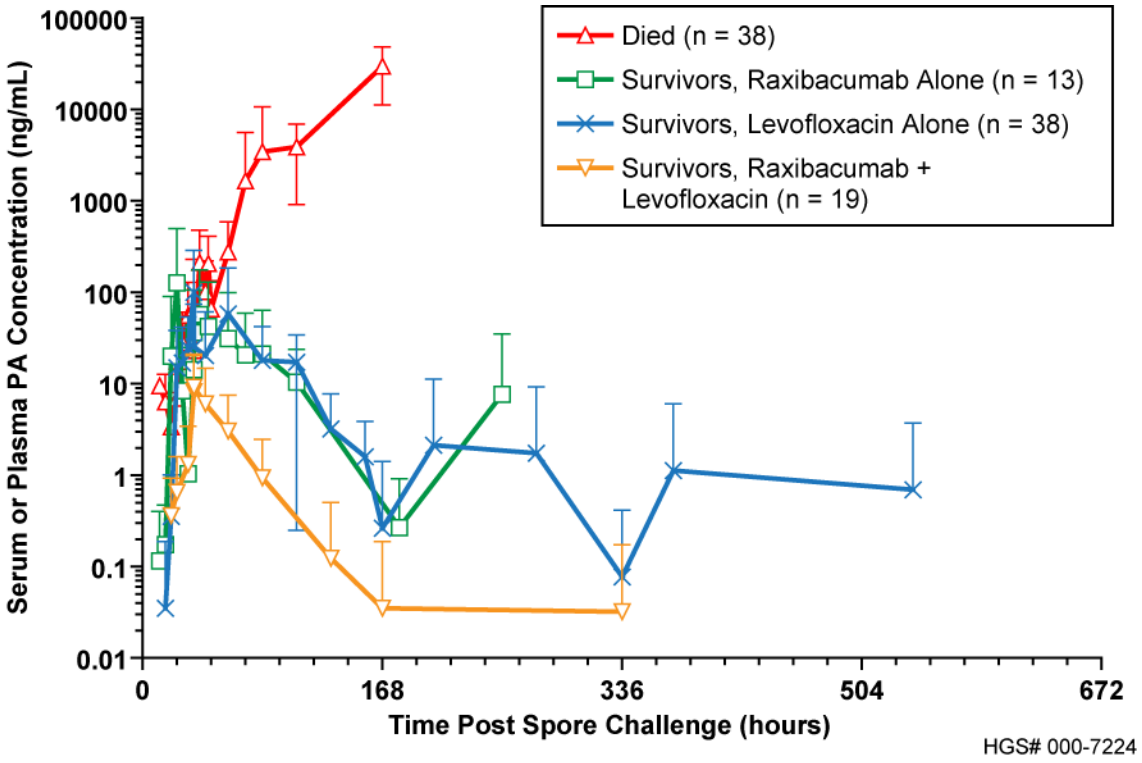

Figure 13-61 Serum/plasma PA concentration-time profiles for rabbits that died vs those that survived (mean ± SD)

For the purpose of preparing this figure, results for terminal specimens from the rabbits that died were plotted as 168 h. PA was measured in serum samples for the raxibacumab monotherapy study (682-G005758) and in plasma for the levofloxacin/raxibacumab combination study (781-G923701) and levofloxacin pilot study (723-G005835). Animals that died received neither raxibacumab nor levofloxacin.

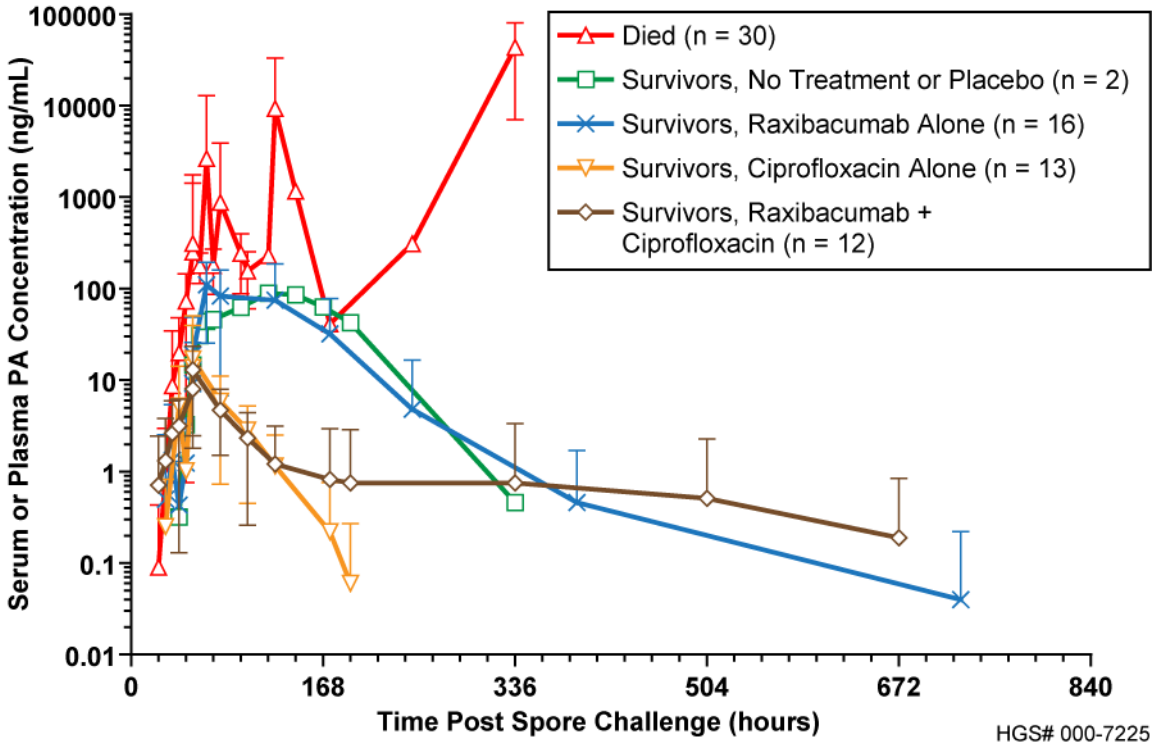

Figure 13-62 Serum PA concentration-time profiles for monkeys that died vs those that survived (mean ± SD)

PA was measured in serum samples for the raxibacumab monotherapy study (682-G005758) and for the ciprofloxacin/raxibacumab combination study (789-G923702). Animals that died received neither raxibacumab nor levofloxacin.

The raxibacumab C_{max} and $AUC_{0-\infty}$ for animals that died vs those that survived are summarized in Table 13-72. There is no consistent pattern of increased or decreased raxibacumab exposure in animals that died vs those that survived, and the differences in raxibacumab C_{max} and $AUC_{0-\infty}$ between animals that died and those that survived are not statistically significant. On the basis of C_{max} and $AUC_{0-\infty}$, there is not a clear cut relationship between raxibacumab exposure and survival outcome. Hence, it is not possible to define a minimal effective exposure (C_{max} and $AUC_{0-\infty}$) for raxibacumab.

However, in the pivotal and confirmatory efficacy studies, survival rates for the 40 mg/kg treatment groups were numerically superior to those for the 20 mg/kg treatment groups. In both rabbits and monkeys, there was a statistically significant increasing trend in survival across the placebo, 20 mg/kg raxibacumab, and 40 mg/kg raxibacumab groups. The relative survival rate in the 40 mg/kg raxibacumab group was 160% and 129% compared with the 20 mg/kg raxibacumab group for rabbits and monkeys, respectively, although these differences did not achieve statistically significance.

Given the high mortality associated with anthrax, the inability to define a minimal effective C_{max} or $AUC_{0-\infty}$, and the raxibacumab mechanism of action (binding of PA), it seems reasonable to conclude that prudent selection of a human raxibacumab dose should target maximizing serum raxibacumab concentrations as soon as practical after initiation of treatment.

Table 13-72 C_{max} and $AUC_{0-\infty}$ for single IV raxibacumab doses for animals that survived and those that died (mean, 95% CI)

	Study	Raxibacumab Dose (mg/kg)	Survival Status	C_{max} (µg/mL)	$AUC_{0-\infty}$ (µg·day/mL)
Rabbit	682-G005758	20	Died (n = 12)	460 (420,499)	1722 (1604,1841)
			Survived (n = 5)	460 (397,524)	1711 (1311,2112)
Rabbit	682-G005758	40	Died (n = 11)	920 (825,1015)	3366 (3169,3562)
			Survived (n = 8)	918 (830,1005)	3504 (2963,4045)
Rabbit	781-G923701	40[1]	Died (n = 1)	1067	4524
			Survived (n = 17)	929 (877,982)	4439 (4014,4865)
Monkey	724-G005829	20	Died (n = 7)	506 (398,613)	3773 (2864,4682)
			Survived (n = 7)	475 (429,522)	3379 (2773,3985)
Monkey	724-G005829	40	Died (n = 5)	897 (749,1044)	6395 (4343,8447)
			Survived (n = 9)	1042 (905,1178)	6544 (4699,8389)
Monkey	789-G923702	40[2]	Died (n = 2)	1167 (1023, 1311)	7006 (3572, 10440)
			Survived (n = 12)	1067 (967,1167)	9903 (8455,11351)

Abbreviations: C_{max}, maximum serum drug concentration; $AUC_{0-\infty}$, area under the serum drug concentration-time curve from time 0 to infinite time.

1 Raxibacumab was administered with levofloxacin.

2 Raxibacumab was administered with ciprofloxacin.

3 Mean and range is presented.

In the rabbit and monkey efficacy studies, animals that survived developed measurable anti-PA antibody concentrations and TNA titers by 14 to 28 days post spore challenge (Reports 723-G005835, 781-G923701, 685-G005762, 724-G005829, and 789-G923702). A rechallenge study in monkeys (374-N006090) showed that the innate immune response was sufficient to protect the animals from a 2nd *B. anthracis* spore challenge approximately 11 months after the 1st challenge. Humans exposed to *B. anthracis* would also be expected to develop anti-PA antibody and TNA if mortality due to PA toxemia during the 1st month post-exposure can be avoided. For the 6 surviving subjects from the 2001 anthrax attack, anti-PA was first detected up to 28 days after likely exposure (Quinn et al, 2004), and in volunteers administered anthrax vaccine adsorbed (AVA) on different schedules, peak anti-PA concentrations occurred 3 to 6 weeks after the 1st vaccine dose (Pittman et al, 2002). Hence, another characteristic of an efficacious human raxibacumab dose is that it maintains protective systemic raxibacumab exposure for a sufficient duration after administration to allow the innate immune response to PA to develop.

Overall, the 2 characteristics of an effective human raxibacumab dose are a sufficiently high C_{max} and at least 28 days duration of protective serum raxibacumab levels. These characteristics were assessed for the single 40 mg/kg IV infusion raxibacumab dose in humans by determining the proportion of human subjects for whom raxibacumab exposure can be considered as protective for survival against inhalation anthrax. This was estimated in the following 2 different ways:

- Human raxibacumab PK results were compared with those for *B. anthracis* spore-challenged rabbits and monkeys that survived following treatment with 40 mg/kg raxibacumab administered IV. The proportion of humans expected to attain a C_{max} or $AUC_{0-\infty}$ greater than the lowest C_{max} or $AUC_{0-\infty}$ values for a surviving rabbit or monkey was then determined.
- Human raxibacumab PK results were used to calculate the predicted percent of PA bound by raxibacumab at selected times post-dose, based on the K_d for raxibacumab determined in vitro. For this analysis, the highest observed serum/plasma PA concentrations prior to death in a rabbit or monkey were used as the expected human serum PA concentrations. The percent of PA bound was based on the lower 90% CI bound for the predicted human serum raxibacumab concentration-time profile (thus ensuring 95% of human subjects would have concentrations equal to or greater than that value).

13.17.4 Raxibacumab Exposure in Humans Compared with Animals That Survived *B. anthracis* Spore Challenge

Comparisons of human raxibacumab PK with raxibacumab PK for rabbits and monkeys are provided in Table 13-73 and Table 13-74, respectively. For all 3 species, V_1 approximates the plasma volume, with the result that C_{max} for a given dose is similar across the species (918 to 1067 µg/mL at 40 mg/kg). It should be noted that C_{max} does not appear to differ between healthy animals and animals with anthrax. In spore-challenged rabbits and monkeys administered raxibacumab only, CL was increased relative to healthy animals, whereas for animals administered raxibacumab with an antibiotic, CL was intermediate to that for healthy animals. These observations suggest that anthrax disease state affects raxibacumab CL. The lesser impact observed when raxibacumab was administered with antibiotic may be related to the rapid sterilization of bacteremia by the antibiotic, which may lead to earlier normalization of organ function, and hence, normalization of raxibacumab CL. CL was faster for rabbits than for monkeys and humans, and human raxibacumab CL was slowest. Most importantly, the differences in CL among species result in an $AUC_{0-\infty}$ for a 40 mg/kg dose to humans that is nearly 3-fold greater than that for monkeys with anthrax, and more than 5-fold greater than that for rabbits with anthrax. Related to this, $t_{1/2,\beta}$ was fastest in rabbits, intermediate in monkeys, and slowest in humans. This implies that for a given dose size, protective raxibacumab concentrations would be maintained for a longer duration in humans than in either rabbits or monkeys.

Comparison of the raxibacumab PK results observed for rabbits and monkeys in the therapeutic intervention efficacy studies with the PK in healthy humans shows that C_{max} is similar for a 40 mg/kg dose across species. Given the raxibacumab mechanism of action (binding PA to block lethal effects of toxemia, it is reasonable to expect that attaining similar

C_{max} should result in similar efficacy in the therapeutic intervention setting. Hence, a 40 mg/kg dose administered to humans should have efficacy similar to that observed in the animal efficacy studies for that dose.

Examination of individual subject results showed that after a 40 mg/kg raxibacumab IV dose, the lowest C_{max} and $AUC_{0-\infty}$ in a human subject were 589 µg/mL and 8720 µg·day/mL, respectively, while the lowest observed C_{max} and $AUC_{0-\infty}$ for surviving spore-challenged rabbits were 405 µg/mL and 1411 µg·day/mL, respectively, and were 385 µg/mL and 2499 µg·day/mL, respectively, for surviving spore-challenged monkeys. This indicates that a 40 mg/kg dose to humans can be expected to provide exposure associated with survival for virtually all subjects. Given the linearity of human raxibacumab PK, the minimum C_{max} and $AUC_{0-\infty}$ for a 20 mg/kg IV raxibacumab dose in a human subject would be about 295 µg/mL and 4360 µg·day/mL, respectively. While the extrapolated minimum $AUC_{0-\infty}$ for a 20 mg/kg dose to humans exceeds the lowest $AUC_{0-\infty}$ associated with survival in spore-challenged rabbits or monkeys, the minimum C_{max} is lower than the lowest C_{max} associated with survival in rabbits or monkeys. This finding indicates that a proportion of human subjects administered a 20 mg/kg dose would be at risk of not attaining protective serum raxibacumab levels, unlike a 40 mg/kg dose.

Table 13-73 Raxibacumab PK in rabbits and in healthy humans (mean ± SD)

	Healthy Rabbits AB50409.INF.0.016		Spore-Challenged Rabbits That Survived Report 682-G005758		Spore-Challenged Rabbits That Survived Report 781-G923701	Healthy Humans Population Analysis	
	1 mg/kg (n = 4)	10 mg/kg (n = 4)	20 mg/kg[1] (n = 5)	40 mg/kg[1] (n = 8)	40 mg/kg[2] (n = 18)	40 mg/kg[1] (n = 322)	20 mg/kg[3]
C_{max} (µg/mL)	26 ± 1	276 ± 19	460 ± 51	918 ± 105	929 ± 106	960 ± 164	480 ± 82
$AUC_{0-\infty}$ (µg·day/mL)	174 ± 60	1518 ± 408	1711 ± 323	3504 ± 647	4439 ± 856	16667 ± 3198	8334 ± 1599
$t_{1/2,\alpha}$ (h)	0.40 ± 0.22	0.24 ± 0.05	0.23 ± 0.04	0.26 ± 0.05	0.09 ± 0.01	1.76 ± 0.36	1.76 ± 0.36
$t_{1/2,\beta}$ (h)	8.7 ± 4.4	6.9 ± 2.7	3.86 ± 1.11	4.15 ± 1.22	4.58 ± 0.70	22.35 ± 4.04	22.35 ± 4.04
MRT (h)	12.0 ± 6.0	9.7 ± 3.7	5.41 ± 1.54	5.79 ± 1.69	6.55 ± 0.99	30.09 ± 5.76	30.09 ± 5.76
CL (mL/kg/day)	6.2 ± 1.8	6.9 ± 1.8	11.96 ± 2.36	11.60 ± 2.42	9.35 ± 1.91	2.49 ± 0.49	2.49 ± 0.49
CLD_2 (mL/kg/day)	NA	NA	37.18 ± 1.31	36.73 ± 2.12	86.38 ± 6.74	6.56 ± 0.91	6.56 ± 0.91
V_1 (mL/kg)	39.0 ± 2.1	36.4 ± 2.5	43.56 ± 4.21	43.22 ± 4.53	43.62 ± 5.49	42.86 ± 7.28	42.86 ± 7.28
V_2 (mL/kg)	NA	NA	18.34 ± 4.32	20.70 ± 4.55	16.11 ± 3.16	30.06 ± 4.34	30.06 ± 4.34
V_{ss} (mL/kg)	66.9 ± 9.9	63.2 ± 15.4	61.90 ± 6.45	63.92 ± 7.54	59.73 ± 6.34	72.92 ± 10.07	72.92 ± 10.07

Abbreviations: C_{max}, maximum serum drug concentration; $AUC_{0-\infty}$, area under the serum drug concentration-time curve from time 0 to infinite time; $t_{1/2,\alpha}$, elimination half-life for the 1st phase; $t_{1/2,\beta}$, elimination half-life for the 2nd (terminal) phase; MRT, mean residence time; CL, clearance; CLD_2, intercompartmental clearance; V_1, volume of distribution for the central compartment; V_2, volume of distribution for the peripheral compartment; V_{ss}, volume of distribution at steady-state; NA, not available.

1 Based on individual post hoc estimates.

2 Based on individual post hoc estimates; raxibacumab was administered with levofloxacin.

3 Extrapolated values, assuming linear PK.

Table 13-74 Raxibacumab PK in monkeys and in healthy humans (mean ± SD)

	Healthy Monkeys AB50409.INF.0.017		Spore-Challenged Monkeys That Survived			Healthy Humans	
			Report 724-G005829		Report 789-G923702	Population Analysis	
	1 mg/kg (n = 4)	10 mg/kg (n = 4)	20 mg/kg[1] (n = 7)	40 mg/kg[1] (n = 9)	40 mg/kg[2] (n = 12)	40 mg/kg[1] (n = 322)	20 mg/kg[3]
C_{max} (µg/mL)	29 ± 6	262 ± 30	475 ± 50	1042 ± 177	1067 ± 158	960 ± 164	480 ± 82
$AUC_{0-\infty}$ (µg·day/mL)	267 ± 91	2030 ± 172	3379 ± 655	6544 ± 2400	9903 ± 2279	16667 ± 3198	8334 ± 1599
$t_{1/2,\alpha}$ (h)	1.10 ± 0.84	0.69 ± 0.53	0.69 ± 0.10	0.68 ± 0.14	0.64 ± 0.12	1.76 ± 0.36	1.76 ± 0.36
$t_{1/2,\beta}$ (h)	15.8 ± 4.1	11.8 ± 1.9	10.80 ± 1.79	9.95 ± 2.48	15.27 ± 4.53	22.35 ± 4.04	22.35 ± 4.04
MRT (h)	19.8 ± 4.3	15.8 ± 1.7	14.38 ± 2.64	13.06 ± 3.53	20.78 ± 6.33	30.09 ± 5.76	30.09 ± 5.76
CL (mL/kg/day)	4.1 ± 1.4	5.0 ± 0.4	6.09 ± 1.15	6.64 ± 2.00	4.25 ± 1.05	2.49 ± 0.49	2.49 ± 0.49
CLD_2 (mL/kg/day)	NA	NA	19.95 ± 3.11	19.03 ± 2.97	21.34 ± 2.98	6.56 ± 0.91	6.56 ± 0.91
V_1 (mL/kg)	36.0 ± 7.8	38.6 ± 4.3	42.41 ± 4.81	38.80 ± 6.05	38.18 ± 5.14	42.86 ± 7.28	42.86 ± 7.28
V_2 (mL/kg)	NA	NA	43.04 ± 2.60	42.07 ± 5.83	44.88 ± 7.58	30.06 ± 4.34	30.06 ± 4.34
V_{ss} (mL/kg)	78.8 ± 23.7	78.0 ± 8.3	85.45 ± 6.90	80.87 ± 10.40	83.06 ± 10.08	72.92 ± 10.07	72.92 ± 10.07

Abbreviations: C_{max}, maximum serum drug concentration; $AUC_{0-\infty}$, area under the serum drug concentration-time curve from time 0 to infinite time; $t_{1/2,\alpha}$, elimination half-life for the 1st phase; $t_{1/2,\beta}$, elimination half-life for the 2nd (terminal) phase; MRT, mean residence time; CL, clearance; CLD_2, intercompartmental clearance; V_1, volume of distribution for the central compartment; V_2, volume of distribution for the peripheral compartment; V_{ss}, volume of distribution at steady-state; NA, not available.

1 Based on individual post hoc estimates.

2 Based on individual post hoc estimates; raxibacumab was administered with ciprofloxacin.

3 Extrapolated values, assuming linear PK.

13.17.5 PA Binding by Human Raxibacumab Concentrations

In vitro, the dissocation equilibrium constant (K_d) for raxibacumab was estimated to be 2.78 nM. Using the K_d, the percentage of plasma PA bound following raxibacumab dosing can be calculated from the following equation (Eq. 8, based on Metzler, 1977).

$$\%Bound = 100\left(K_d^{-1}C_{raxi} \Big/ 1 + K_d^{-1}C_{raxi}\right)$$

Equation 1, where C_{raxi} is the plasma raxibacumab concentration, expressed as nM.

Based on the median human serum raxibacumab concentration-time profile and lower 90% prediction interval bound profile for a single 40 mg/kg IV raxibacumab dose, the percentage of PA that could be bound were calculated and are summarized in Table 13-75. This table also includes the percentage of PA that would be bound for a single 20 mg/kg dose based on extrapolation from the 40 mg/kg dose results. A single IV 40 mg/kg raxibacumab dose to humans can be expected to produce serum drug concentrations high enough to bind at least 99% of serum PA for at least 42 days post-dose in at least 95% of the subjects. Greater than 99% of PA would be bound following a 20 mg/kg dose; however, that level of binding would not be maintained through 42 days.

Table 13-75 Serum raxibacumab concentrations in healthy humans and PA binding

	Serum Raxibacumab Concentrations at:											
	20 mg/kg						40 mg/kg					
	Median			Lower 90% PI			Median			Lower 90% PI		
Time (Days)	µg/mL	nM	% Bound for PA	µg/mL	nM	% Bound for PA	µg/mL	nM	% Bound for PA	µg/mL	nM	% Bound for PA
End of Infusion	452	3115	99.9	317	2185	99.9	903	6230	100.0	634	4370	99.9
1	326	2251	99.9	243	1672	99.9	653	4502	99.9	485	3345	99.9
14	142	978	99.7	99	683	99.6	283	1955	99.9	198	1367	99.8
28	90	623	99.6	55	380	99.4	181	1246	99.8	110	759	99.7
42	58	403	99.4	30	203	98.8	117	806	99.7	59	407	99.4
56	38	260	99.1	15	104	97.7	75	520	99.5	30	209	98.8

The highest observed serum/plasma PA concentrations observed prior to death in control rabbits and monkeys (ie, not administered raxibacumab or antibiotic) that died were 24752 and 63096 ng/mL (298 and 760 nM), respectively. In both instances, the concentration represents the last collection time prior to death. PA concentrations for terminal specimens collected after death were not considered due to possible post-mortem changes, and also because the intent was to evaluate relative concentrations of PA and raxibacumab for therapeutic intervention prior to death. It is assumed that these highest observed serum/plasma PA concentrations in a rabbit and monkey represent the highest levels that might be encountered in a human subject to be treated with raxibacumab. Figure 13-63 illustrates the

median and 90% prediction interval serum raxibacumab profiles for a 40 mg/kg single IV dose, expressed as nM concentrations, overlaid with the expected highest PA concentrations to be encountered, expressed as nM. The corresponding illustration for a 20 mg/kg raxibacumab dose to humans is provided in Figure 13-64. As shown in Figure 13-63, following a 40 mg/kg dose, serum raxibacumab levels are equimolar to or greater than the highest expected PA levels for 28 or 48 days, using PA levels from monkeys and rabbits, respectively. In contrast, following a 20 mg/kg dose, serum raxibacumab levels were equimolar to or greater than the highest expected PA levels for 11 to 33 days. Of note, for the 6 surviving subjects from the 2001 anthrax attack, anti-PA IgG was first detected up to 28 days after likely exposure (Quinn et al, 2004), and in volunteers administered AVA, peak anti-PA IgG concentrations occurred 3 to 6 weeks after the 1st vaccine dose (Pittman et al , 2002). Since it would be desirable for a human raxibacumab dose to provide protective serum drug levels for at least 28 days to ensure an innate immune response can develop, a 40 mg/kg dose would be superior to a 20 mg/kg dose.

Overall, based on raxibacumab PK in rabbits, monkeys, and humans, as well as serum/plasma PA in *B. anthracis* spore-challenged rabbits and monkeys, it appears that a single 40 mg/kg raxibacumab dose in humans should have efficacy for the treatment of inhalation anthrax comparable to that observed in the nonclinical therapeutic efficacy studies. Evaluation of a 20 mg/kg dose for humans indicates that it would likely be inferior to the 40 mg/kg dose, in that a 20 mg/kg dose would not provide exposures greater than or equal to those shown to be associated with survival in nonclinical studies for all subjects, and may not provide sufficient duration of protection for innate immunity to develop. In contrast, a 40 mg/kg dose to humans should result in virtually all subjects attaining exposures associated with survival in the animal studies, with a duration of protective levels of at least 28 days for the majority of subjects, allowing the development of innate immunity.

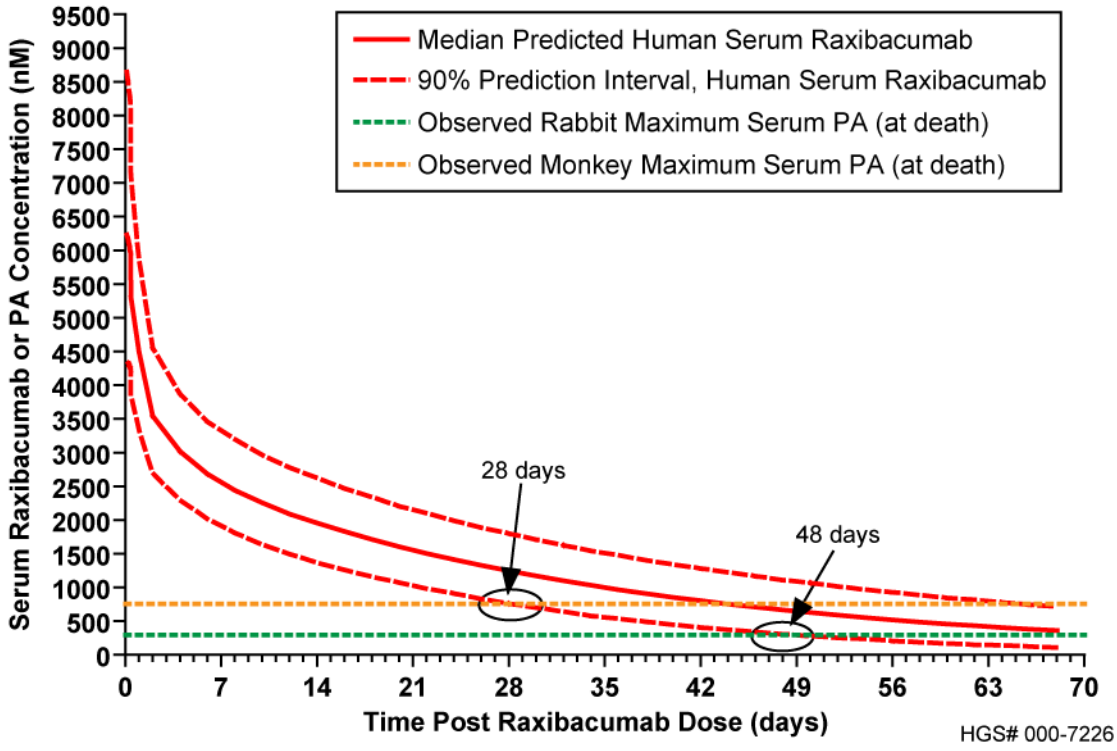

Figure 13-63 **Median and 90% prediction interval serum raxibacumab concentration-time profiles for a single 40 mg/kg raxibacumab IV infusion dose, relative to the highest expected serum/plasma PA concentrations**

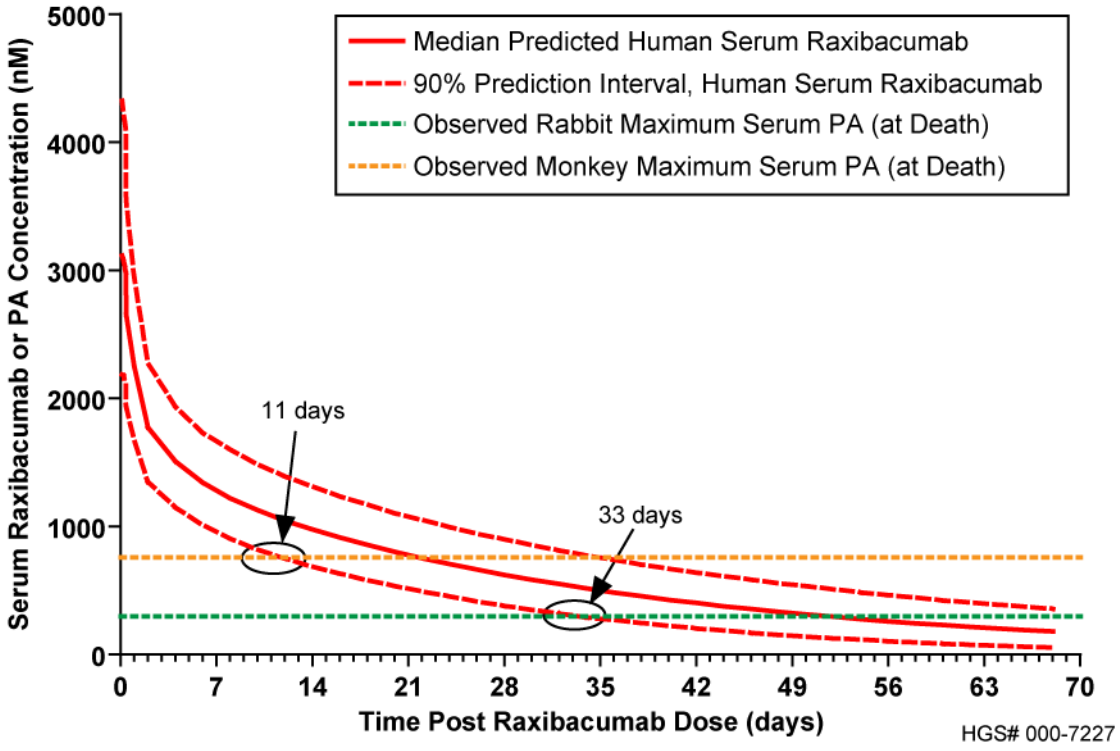

Figure 13-64 **Median and 90% prediction interval serum raxibacumab concentration-time profiles for a single 20 mg/kg raxibacumab IV infusion dose, relative to the highest expected serum/plasma PA concentrations**